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CONTENTS

	PAGE
RELATION OF BLOOD CELLS TO CONNECTIVE TISSUES AND ENDOTHELIUM. *Alexander A. Maximow*	533
THE ETIOLOGY OF DENTAL CARIES. *John Albert Marshall*	564
A REVIEW OF ACUTE EXPERIMENTAL NEPHRITIS. *Wm. deB. MacNider*	595
RECENT ADVANCES IN BIOLUMINESCENCE. *E. Newton Harvey*	639

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PHYSIOLOGICAL REVIEWS

VOL. IV OCTOBER, 1924 No. 4

RELATION OF BLOOD CELLS TO CONNECTIVE TISSUES AND ENDOTHELIUM[1]

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In the normal adult organism the cells of the blood seem to be very independent from the connective tissue, but in reality the relations between these two types of elements are so intimate that no distinct line can be drawn between them. This is perfectly obvious, if we follow the embryonic histogenesis of blood and connective tissue. It is equally manifested in the adult, under pathological conditions, particularly in inflammation or in tissue cultures.

In the very young embryo, after the formation of the germ layers, the mesenchyme, the embryonic connective tissue, arises from various places in the mesoderm, especially from the sclerotomes. The elements of the peripheral mesoblast, which are, in fact, also mesenchymal elements, form the blood islands; in the higher vertebrates and the selachians in the extra-embryonic area, in the teleosts and amphibians in the body of the embyro itself (80). Neither ectoderm nor entoderm plays any rôle in the production of the island cells.

The cells of the blood islands differentiate in two directions—the peripheral form the wall of the first blood vessels, the first endothelium; the central become free and float in the liquid, accumulating in the lumen of the endothelial tubes, the blood plasma; they represent spherical, isolated mesenchymal elements, are all alike and have to be called primitive blood cells (59). Thus the system of the yolk vessels arises.

During the transformation of the solid blood islands into the hollow vessels the first blood plasma, secreted by the island cells, appears and accumulates between the latter in the form of intercellular vacuoles.

[1] Read in abbreviated form at the symposium on blood, before the American Association of Anatomists, at the fortieth annual session in Buffalo, April 19, 1924.

Thus, the lumen of the first blood vessels is to be looked upon as an intercellular space. In the thin cellular strands, connecting the blood islands with each other, the vacuoles may first appear intracellularly and secondarily fuse with the plasma drops, arising between the cells. Cell degeneration and liquefaction does not play any appreciable rôle in the process, although occasionally a few degenerating cells may be found in the islands.

Shortly after the formation of the blood islands in the area opaca vessels appear in the area pellucida; in the body of the embryo both heart and vessels arise. At first, they are all disconnected, irregular, blind cavities, lined with flattened mesenchymal cells, the endothelium, but very soon they fuse with each other and with the vessels of the area opaca. The majority of them contain only blood plasma, but no primitive blood cells. Typical blood island formation can be found in the body of the embryo, but it never reaches any considerable development (59), (81).

Thus the various parts of the circulatory system, arising independently and at a slightly different time from the mesenchyme in the different regions, quickly come into contact with each other and a continuous system of endothelial tubes is established.

According to the old angioblast theory of His (37), the circulatory system of the higher vertebrate embryo, i.e., both the walls of the blood vessels and the blood cells, originates in the extraembryonic region in the way just mentioned, from the so-called parablast, in the form of an independent, distinct unit, the angioblast. The vessels in the area pellucida and in the body arise through ingrowth of angioblastic elements out of the area opaca.

Although, as we shall see, the endothelium, in later stages, indeed seems to become a highly independent, specifically differentiated tissue, yet the angioblast theory cannot be maintained. The heart and the vessels in the body of the embyro and in the area pellucida arise, as has been conclusively shown by many investigators (81), (82), not through ingrowth from the area opaca, but in situ, from local mesenchyme cells, only a little later, than the peripheral vessels in the area opaca. Besides, the theory of simultaneous formation of endothelium and blood cells from the same common source can be applied only to the origin of the first, primitive blood vessels. Later, even in embryonic life, the blood cells can originate everywhere in the body from mesenchymal elements and multiply quite independently from the blood vessels. The so called "vasoformative cells" of Ranvier (75), which were thought to produce, even in newborn animals, blood vessels and blood corpuscles simultaneously, have now received, as we shall see below, a totally different explanation.

Thus, the idea of the existence of specific elements, responsible for production of endothelium and blood cells in the later stages of embryonic life has to be abandoned and the term angioblast becomes superfluous.

The common, fixed, stellate mesenchyme cell (fig. 1, *a*), the primitive endothelium, *b*, and the primitive blood cells, *c*, *1*, during a certain short stage of development, represent the only mesenchymal elements available in the body of the embryo and in the surrounding areas as well. Originating from the same source, the primitive mesenchyme, they are closely related with each other. As I have found for the rabbit (59), during a certain very short time after the dissolution of the blood islands, the endothelium of the yolk vessels furnishes a small additional quantity of primitive blood cells, *d*. Endothelial cells bulge into the lumen and become free. In other mammals, for instance in the sheep, according to Bonnet (5), perhaps the majority of the primitive blood cells may originate in this way. But, nevertheless, the prospective potencies of the three cell types mentioned are no longer identical. The fixed mesenchyme cells cannot produce any more blood islands or primitive blood cells; their transformation into endothelium also continues, as we have seen, only for a very short time in the area pellucida and in the embryonic body (*e*) and very soon ceases.

The primitive blood cells in the lumen of the yolk vessels (fig. 1, *1*) differentiate in two directions (59). The majority produces through differentiating proliferation peculiar hemoglobin containing elements, the primary erythroblasts and erythrocytes, which for a certain time serve as oxygen carriers, but gradually die out and disappear, *5*. The rest remain as colorless, ameboid, basophilic cells (fig. 1, cell lineage *f*, *2*).

This differentiation of the primitive blood cells in two directions, one represented by primitive, temporary red corpuscles, the other by elements, remaining colorless, and keeping the undifferentiated condition together with the full amount of hematopoietic potencies, is one of the fundamental facts in modern hematology. It has been positively stated for the embryonic development of the selachians (64), the amphibians (48), the reptiles (13), the birds (12) and the mammals (59).

What is the nature of the free colorless, undifferentiated cells in the lumen of the yolk vessels (fig. 1, cell lineage *f*, *2*)? They are ameboid, spherical mesenchyme cells, floating in the blood plasma. When the circulation begins, they at once are carried, together with the primitive erythroblasts, but at first only in very small quantities, into the general

blood stream. It is quite logical, therefore, to look upon them as the first white blood corpuscles, the first leukocytes of the embryo. Histologically they resemble typical large lymphocytes of the adult organism. This is the reason why I gave them this name in 1907. I was perfectly aware (59, p. 472), that this term is somewhat incongruous as, at the time of their appearance, there is no lymphoid tissue as yet, but I did not want to complicate unnecessarily the hematological terminology. We shall see that these "large lymphocytes" are the common stem cells of all the other blood elements in the embryo and in the adult. The most appropriate name for them might be, perhaps, "hemocytoblasts" (Ferrata), which means "cells producing blood cells."

The hemocytoblasts in the yolk vessels cannot be looked upon merely as primitive blood cells, persisting in an unchanged condition, as Jolly (38) believes, because their prospective potencies are different—they are unable to produce primitive erythroblasts.

In a series of recent publications, Ferrata (29), di Guglielmo (33), Lambin (45) and others are claiming that the hemocytoblasts in the vessels of the area opaca are not white cells at all, but young, highly basophilic, transitional forms from the primitive blood cells to the primitive erythroblasts. Thus, the old idea of the transformation of all free blood island cells without exception into red blood cells is again being resuscitated.

I believe that this mistake is partly due to the extensive use of unsuitable technique, expecially dry smears, which always causes in embryonic material grave artifacts. In fresh preparations the hemocytoblasts are colorless and ameboid; the erythroblasts, even the youngest ones, motionless, regularly spherical and of a more or less distinct yellow color. In preparations duly fixed and stained, the nuclei of the hemocytoblasts are quite different from the nuclei of the cells of the hemoglobin series. It is easy to see that the hemocytoblasts in the yolk vessels are the same cells, as the first "lymphoid" wandering cells of the extravascular mesenchyme, and nobody will believe the latter to be young forms of erythroblasts. Especially in the lower vertebrates, for instance in the area vasculosa of the selachian embryos, the identity of the highly ameboid intravascular and extravascular "large lymphocytes" or hemocytoblasts is established beyond doubt.

Simultaneously with the transformation of the primitive blood cells, which were not used up in the formation of primitive erythroblasts, into hemocytoblasts, the endothelium of the yolk vessels still continues for a certain time to produce new free cells; they are partly of hemocy-

toblastic character and join the intravascular hemocytoblasts (fig.1, *g*, *2*). But most of these new intravascular cells of endothelial origin are of a different type —they have abundant, highly ameboid, pale, vacuolated protoplasm and a small, irregular nucleus (fig. 1, *h*, *3*, *4*). They are active phagocytes and thus in 1907 I gave them the name of endothelial phagocytes (59, p. 493). However, no sharp line of distinction can be drawn between them and the large basophilic hemocytoblasts or "large lymphocytes"; transitional forms are always present (fig. 1, *i*). The existence of these phagocytic cells of endothelial origin has been confirmed by Sabin (81).

Their appearance in the yolk vessels and their close relationship with the hemocytoblasts are important facts. These cells correspond to the "histioid or histiogenous wandering cells" of the mesenchyme, described below, and are to be looked upon as the first specimens of the "histiocytic" (fig. 1, *h*, *3*) and "monocytic" (*h*, *4*) cell types, playing such an important rôle in the future life of the organism. They remain during the whole life as the chief connecting link between the connective tissue and the blood.

A certain, very small number of the endothelial phagocytes may pass, together with the basophilic hemocytoblasts, into the general circulation. However, there is no sufficient reason to admit the exclusive presence of numerous white blood cells of endothelial origin (hemohistioblasts of the Italian school) in the embryonic circulation (33). Here again defective technique is responsible—by puncturing the embryonic heart with a pipette and preparing dry smears one is liable to get numerous elements of undefinable origin and in badly damaged condition into his preparations.

The yolk vessels are the first hematopoietic organ of the vertebrates with meroblastic eggs and of the mammals. In their lumen the hemocytoblasts (fig. 1, *f*, *2*) for the first time display their latent potencies of development. They proliferate and whereas a part of their progeny remains unchanged and may produce megacaryocytes, the majority becomes transformed into secondary erythroblasts and finally into non nucleated erythrocytes (*f*, *6*), which gradually replace the primary erythroblasts and erythrocytes. In the mammals this is the only product of the hemacytoblasts in the yolk sac. In other vertebrates—birds, reptiles, selachians—the hemocytoblasts in the yolk sac wall also produce granulocytes and thrombocytes, and, in the selachians, even small lymphocytes (64). The granulocytes and the small lymphocytes, however, always originate only extravascularly, whereas the secondary erythroblasts appear in the lumen of the vessels as in the mammals.

Thus the fundamental proof of the unitarian theory of hematopoiesis, or the conception that all the different blood elements originate from one common stem cell of lymphoid type, can be easily found in the first hematopoietic organ, the yolk sac wall. According to various external conditions, the hemocytoblast produces different cell lineages with specific characters.

The hemocytoblasts, the large lymphocytes, whose first appearance in the yolk vessels I have just discussed, do not separate from the rest of the mesenchyme once for all. On the contrary—new cells of this type continue to arise from the undifferentiated fixed mesenchyme cells. In the early embryonic stages, just after the establishment of the general circulation, extravascular basophilic lymphocytoid hemocytoblasts appear everywhere in the body of the embryo and in its appendices (fig. 1, *k*). They arise from the common fixed mesenchyme cells in loco through contraction and mobilization (59); they are scattered and creep about in the meshes of the mesenchymal tissue framework and divide mitotically. Saxer (84) was the first to describe them under the name of "primary wandering cells", in 1896. But he believed them to originate in the blood islands and to be brought by the blood stream or in some other way into the body of the embryo.

Simultaneously, or a little later, among the large ameboid basophilic lymphocytoid wandering cells, the hemocytoblasts or large lymphocytes (according to my older terminology), appear the so called "histiogenous" or "histioid" wandering cells—elements with a smaller, darker nucleus and a vacuolated, slightly or non basophilic protoplasm (59, p. 502) (fig. 1, *1*, *3*, *4*). They are identical with the "endothelial phagocytes" or monocytes, mentioned above in the yolk vessels, with the only difference, that here they arise from common mesechyme cells, whereas there they were budding off from the endothelial wall into the lumen. Here, as well as there, they are connected with the basophilic hemocytoblasts by a series of intermediate forms (fig. 1, *m*) and correspond to the mobilized free histiocytes of the adult connective tissue. They are phagocytic and are able to store vital dyes (43).

I wish to emphasize especially that, already in these early stages, the histioid wandering cells, the free histiocytes, cannot be distinctly separated from the basophilic lymphocytes, the hemocytoblasts, neither in the blood of the yolk vessels and in the general circulation, nor in the extravascular mesenchymal tissue. The two cell types mentioned are two modifications of one and the same fundamental cell type, a mesenchymal wandering element, and are endowed with the

same prospective potencies of development. As we shall see below, in the adult organism the hemocytoblasts are free, isolated elements, that remain confined more to the blood and lymph and to the blood forming organs, whereas the histiocytes are mostly concentrated, as fixed elements, in connective tissue and in the stroma of the blood forming organs. But both groups of cells remain for the whole life in continuous intimate intercourse and display their close relationship or identity upon innumerable occasions.

As soon as the hemocytoblasts appear in the body mesenchyme, they (as well as the histioid wandering cells) are seen to produce small extravascular foci of secondary erythroblasts (fig. 1, *k*, *6*) and granular cells—myelocytes and leukocytes of different types (*k*, *7*).

In the mammalian embryo the liver is the second hematopoietic organ. When the liver cell cords push their way into the vascular mesenchyme of the septum transversum, the spaces between them are occupied by large blood sinusoids and only very few mesenchyme cells remain between the endothelium and the liver cells. In these narrow clefts hemocytoblasts appear shortly afterwards (fig. 1, *n*, *2*). They arise partly from the small mesenchyme cells just mentioned, which still can be found between the endothelium of the sinusoids and the glandular tubes (59), partly, perhaps, from the endothelial cells themselves (67), which in the liver, as we shall see, have a peculiar character and never lose their embryonic potencies.

The hemocytoblasts rapidly proliferate in the liver (fig. 1, *o*, *2*); some of them together with a few histiocytes and monocytes (*n*, *3*, *4*) perhaps may continue to arise de novo from fixed cells, probably the endothelium (*n*, *2*). They produce in the usual way, through differentiating proliferation, secondary erythroblasts (*n*, *o*, *6*), granulocytes (*n*, *o*, *7*) and megacaryocytes and thus the liver of the mammalian embryo becomes for a long period a blood forming organ. It is important to note that in the embryonic mammalian liver the erythropoiesis as well as the granulopoiesis both occur extravascularly, although, of course, the walls of the sinusoids are supposed to be not a continuous endothelial membrane, but rather a reticulum of peculiar mesenchyme cells (67).

The bone marrow is the third and the permanent hematopoietic organ of the mammals; in the second half of the embryonic life it gradually takes up the work of the liver which eventually loses all of its hematopoietic constituent parts.

The cartilage of the bone primordia begins to be resorbed in certain places by mesenchyme (fig. 1, *p*), originating in the perichondrium. Here again, in connection with and during this resorption, the mesenchyme cells show excessive activity and produce, through contraction, mobilization and isolation, hemocytoblasts with the structural character of large lymphocytes (*p*, *2*), and pale histioid wandering cells (*q*, *3*) (61). The endothelium of the blood vessels, on the contrary, does not play any visible rôle in this respect. The neoformation of the hemocytoblasts and the histioid wandering cells from fixed mesenchyme elements continues here, at least potentially, during the whole life (*q*, *2*, *3*). Besides, the once formed hemocytoblasts proliferate prodigiously (*r*, *2*) and, in exactly the same way as in the liver, differentiate into erythroblasts (*p*, *q*, *r*, *6*), myelocytes (*p*, *q*, *r*, *7*) and megacaryocytes. In this way, the myeloid tissue of the bone marrow is formed. Being first loosely scattered among the common fixed mesenchyme cells, the round free myeloid cells, gradually increasing in number, finally reduce the fixed elements to a very scant reticular stroma.

As regards the general, diffuse mesenchyme of the embryonic body, it consists in the middle of the embryonic life of two kinds of cells—the fixed, spindle-and star-shaped cells (fig. 1, *a*) and the very numerous wandering elements. The latter continue on the one hand to arise from fixed cells (*t*, *u*), on the other to proliferate independently. Most of them belong now to the histioid type (*u*, *3*), the lymphoid elements (*t*, *2*) being scarce.

The majority of the fixed cells produce now the fibrillar intercellular substance; they differentiate into fibroblasts and lose their manifold prospective potencies, especially the capacity of producing lymphoid or histioid wandering cells.

But this special fibroblastic differentiation does not affect all the fixed cells. The mesenchyme splits into two cell lineages; the one being the fibroblasts, irreversibly transformed into collagen producing elements (fig. 1, *w*), the other, elements remaining for the whole life in an undifferentiated condition, with their embryonic character intact (*x*). The latter keep for ever the capacity of producing lymphoid and histioid wandering cells, hemocytoblasts and histiocytes and, implicitly, all the different types of blood cells. This separation of the mesenchyme into two cell lineages occurs in the diffuse common connective tissue and in the hematopoietic organs, the bone marrow and the lymph nodes, as well. Having begun in the embryo, it remains for the whole life of the organism.

These elements, preserving throughout the whole life their embryonic character, are scattered all over the body (fig. 1, *x*). In the resting condition, in the adult organism, they sometimes cannot very well be distinguished histologically from the fibroblasts, unless special methods, for instance vital staining, be used. This is the case in the serous membranes, especially the omentum, where, perhaps, all the cells in general, including the fibroblasts, remain for always in a more or less embryonic condition (56), (52). They often assume the character of a syncytium, as for instance, in the lymphoid (fig. 1, *aa*) and myeloid (*q*) tissue and in the spleen. In other cases they appear as sharply outlined, polymorphous, flat, spindle shaped or branched elements, often containing inclusions and are then easily distinguishable from the fibroblasts. They may also assume a flat shape and line blood or lymph channels. Finally they often can be found in an active condition—as free, histioid wandering cells, the free histiocytes or the so-called macrophages (*y*).

They are found scattered between the fibroblasts, singly or in small groups, in the common loose connective tissue, where I described them in 1906 under the name of resting wandering cells (56). Evans (27) (28) gives them the name of macrophages. In the serous membranes they are particularly numerous and have been described here by Ranvier (76) as clasmatocytes and by Renaut (77) as rhagiocrine cells. In other places—in the lymph nodes, the bone marrow, the spleen, the wall of the intestine—they are concentrated in great continuous masses, intimately connected with the fibrous reticulin framework and are known as reticular cells. The flat elements, lining the walls of the lymph sinuses in the lymph nodes and the venous sinuses in the spleen, contrary to the opinion of many authors (50), (38), are not true endothelium; they are also reticular cells, having assumed a squamous character (17); the reticular cells of the lymphoid and myeloid tissue are also often called reticulo-endothelial elements (German clinical literature) or hemohistioblasts (29). In the liver they are also flattened cells—the stellate cells of Kupffer—and take part in the lining of the intralobular capillaries. Similar conditions possibly exist in the sinusoids of some endocrine glands, as for instance, in the adrenals.

More than by a peculiar histological structure all these elements are characterized by a series of very important functional properties. Being endowed with ample prospective potencies they can produce, provided external conditions are favorable, hemocytoblasts and different types of blood cells. Under the influence of stimulations, they can

be mobilized and become transformed into free, wandering, proliferating elements of the histioid type, similar to the embryonic histioid wandering cells, described above (fig. 1, *dd*); such histioid wandering cells may for a long time exist and proliferate as an apparently independent cell type of free macrophages (*y*). In the fixed as well as in the free condition the cells are acting as phagocytes and engulf particulate matter. If certain substances in colloidal solution, for instance vital dyes, colloidal metals, etc., reach them, they absorb and store them in their protoplasm in the form of granular inclusions (32), (91), (27), (28). According to Evans, this process may be looked upon as a kind of ultramicroscopic phagocytosis. Lipoids are also often stored in the body of these cells.

It is difficult to choose a suitable general name for these elements of the connective tissue, remaining throughout the whole life in an undifferentiated embryonic condition. They represent a vast cell system, distributed all over the body, over various organs and assuming, according to their position, manifold histological aspects. As we have seen, single parts of this cell system have been described by various investigators under different names, but the idea of the close interrelation or even identity of all these cells, the fact that they form one entity, one vast group of elements with a very prominent function in the body, has made headway slowly.

The first investigator who formulated it clearly was Goldmann (32); he stained them intravitally with pyrrhol blue and called them accordingly "pyrrhol cells." He was followed by Tschaschin (91), by Kiyono (42) and by Evans (27). Kiyono gave them the name "histiocytes." Although this word does not mean anything but "tissue cells," and does not indicate their close relation to the wandering cells, as the term "resting wandering cells" does, or their histological arrangement in the form of stellate, reticular or flattened, endothelium like cells, as the term "reticulo-endothelial system," or their capacity of producing blood tissue, as the term "hemohistioblast," or their size and phagocytic function, as the term "macrophage"—it has the advantage of being short and, besides, it seems to have been largely adopted by the biologists. Therefore I am going to use this term in the following discussion.

Much has been written about the so-called "adventitial cells" of Marchand. They have been described by Marchand (51) in the omentum as elongated elements, accompanying blood vessels; after experimental inflammatory irritations he saw them divide, become

transformed into wandering cells and produce all types of blood cells. It is quite clear that they are common histiocytes, and Marchand himself admits it (52). As the histiocytes do not show any special relations to blood vessels, neither in their origin, nor in arrangement, and are found even in the omentum in places totally devoid of blood vessels, the term "adventitial cells" cannot be considered as appropriate and should be dropped.

Let us now return to the later stages of the embryonic life.

With the formation of the fibrillar intercellular substance, the body mesenchyme gradually becomes transformed into the common loose, irregularly arranged, connective tissue and the vast majority of its cells into fibroblasts (fig. 1, *w*). Among the fibroblasts the histiocytes in resting and in active condition, as histioid wandering cells, are scattered. The basophilic lymphoid hemocytoblasts, the large lymphocytes, are now extremely rare in the common loose tissue. The capacity of producing them becomes more and more restricted and confined to localized areas—the myeloid and the lymphoid tissues (*p*, *q*, *r*, *2*, *aa*, *bb*, *2*).

Of course, the ubiquitous histiocytes, as I pointed out, remain also capable throughout the whole life of producing not only histioid wandering cells, free histiocytes, but also hemocytoblasts, large lymphocytes and, consequently, other types of blood cells. But this does not mean that they always and everywhere are found doing so, even in the embryo. The histiocytes, the resting wandering cells of the common loose connective tissue in older embryos or in the adult, as a rule, do not display their latent hematopoietic potencies. As we shall see later, they are easily mobilized and transformed into polyblasts during inflammation. But for producing lymphocytes and blood cells they must probably meet quite unusual external conditions and this happens, as a rule, only in the omentum (34).

In fact, the hematopoietic activity of the histiocytes becomes exclusively localized for the whole life in the bone marrow and the lymph nodes.

The primordia of the lymph nodes arise relatively late in the embryo, in the neighbourhood of lymph-sacs or lymph-vessels. Small, fairly well outlined areas of mesenchyme become discernible, consisting of numerous small, proliferating, undifferentiated mesenchyme cells, displaying unrestricted hematopoietic potencies (57), (1) (fig. 1, *aa*). They betray from the very beginning their histiocytic nature, in the sense explained above. Whereas at this period in the diffuse mesen-

chyme of the body the production of basophilic hemocytoblasts has been terminated since a long period, in the areas mentioned the mesenchyme cells produce on the one hand histioid wandering cells, free histiocytes (*aa, 3*), on the other hand large quantities of large lymphocytes, i.e., hemocytoblasts (*aa, bb, 2*), medium sized lymphocytes and, finally, small lymphocytes—all crowded in a small space. The small lymphocytes make their first appearance in the primordia of the lymph nodes. It is important to note that here, too, transitions between free histiocytes and lymphocytes are always present (*v*). When the elaboration of the reticulin fibrils begins, a cell lineage of true fibroblasts (*ec*) is split off from the histiocytic syncytium, the latter keeping the unrestricted hematopoietic potencies for ever. But even here, at least in the normal adult, the independent proliferation of lymphocytes largely substitutes their neoformation from fixed histiocytes. Here, as well as in the bone narrow and even in the common diffuse connective tissue, there remains always, on the one hand, a cell lineage of free cells (*bb, 2*), lymphocytes, or histioid wandering cells, *i.e.*, free histiocytes or monocytes, proliferating independently and, on the other hand, a cell lineage of fixed, sometimes syncytial histiocytes, which again are always capable of becoming isolated, free elements (*aa, 2, 3*).

In the later periods of embryonic life the histioid wandering cells, the free histiocytes, scattered in the mesenchyme, partly become quiescent again and transform themselves into the fixed histiocytes of the loose connective tissue, the resting wandering cells of Maximow (*ee*). Thus, the resting wandering cells in the adult connective tissue originate partly directly from the primitive fixed mesenchyme cells and partly through secondary immobilization of wandering elements (1).

A part of the wandering cells of the embryonic mesenchyme becomes transformed into connective tissue mastcells through elaboration of specific granules (1) (fig. 1, *8*). Whereas in the lower vertebrates the connective tissue mastcells are intimately related to the basophilic blood leukocytes and may originate from them through direct transformation after emigration out of the blood vessels, the mastcells and the mastleukocytes of the mammals seem to be independent cell types (62). The first regenerate chiefly through independent mitotic proliferation (56). Downey (16), however, has shown that in the adult they can be also formed anew in the embryonic way, i.e., through elaboration of granules in undifferentiated free cells of lymphocytic or histiocytic type (in lymph nodes).

The transition from the embryonic to postnatal life is not marked by any sudden special changes in the blood or connective tissue. The processes of development and differentiation we have followed in the description given above and in the adjoining diagram, continue without interruption.

In the normal adult organism the hematopoiesis is operated chiefly on the basis of the so-called homoplastic plan. In the bone marrow, for instance, the new myeloid blood cells are mostly formed through proliferation and differentiation of their own specific young forms—the erythrocytes from the erythroblasts, the granulocytes from the respective myelocytes. In the lymphoid tissue the various types of lymphocytes are regenerating through mitotic proliferation of the large and medium sized lymphocytes. In abnormal conditions—after heavy blood losses, in blood diseases—the homoplastic process in the bone marrow may become unsufficient; the hematopoiesis is pushed backward and the heteroplastic formation of myeloid elements may be called forth—the young forms, the erythroblasts and myelocytes are not only proliferating and differentiating to mature forms, but they originate anew, as in the embryo, from primitive, indifferent cells, with a large, clear nucleus and a basophilic, homogenous protoplasm, which histologically are identical with the larger types of lymphocytes in the lymphoid tissue.

I will not go into the details of the controversy between the different unitarian, dualistic and polyphyletic theories of hematopoiesis, of the discussion of the question whether the basophilic indifferent stem cell of the bone marrow is really identical or only similar to the basophilic indifferent large lymphocytes in the lymph nodes.

The unitarian theory, claiming the real identity of the ubiquitous basophilic hemocytoblast, the common stem cell of all blood elements, at the present time seems to dominate in hematology, at least among the biologists and experimenters.

It does not make any difference if this common stem cell really appears, as Downey (21) has recently shown, in two structural modifications, one corresponding to the myeloblast, the other corresponding to the lymphoblast of the dualists. The minute differences of the nuclear structure, seen only in dry smears, might very well depend merely on the differences of external conditions, encountered by the cell in the various places and the various stages of development of the body.

The unitarian theory is based chiefly on investigations in the field of comparative histology and embryology and of experimental biology.

Among the clinicians, however, the dualistic theory is more popular. Operating with minute differences in the nuclear and protoplasmic structure of the normal and pathological blood cells, it supplies them with a convenient, detailed, though somewhat artificial classification of these elements. The basis here is chiefly facts, drawn from the study of dry blood smears.

Whereas the dry smear method gives good opportunity of differentiating the cells circulating in the peripheral blood, it is inadequate for solving the general problems of the origin of blood cells and of their relations to connective tissue. It is deplorable that even in most modern textbooks of clinical hematology, as for instance in the book of Nägeli (68), the biological foundations of morphological hematology, especially the relations of blood elements to connective tissue and endothelium, are to a large extent disregarded or misinterpreted.

At any rate the hematopoiesis in the adult, even under abnormal conditions, in the myeloid and the lymphoid tissue as well, seems to remain largely independent from fixed connective tissue elements and is not pushed farther back than the hemocytoblast. Yet, as we have seen, in the embryo new hemocytoblasts originate from undifferentiated fixed mesenchymal elements. The question therefore is—can hemocytoblasts and, implicitly, blood elements in the adult organism be formed anew in the same way, or not?

That this very rarely, if ever, occurs in the common loose connective tissue, has been pointed out already. The quotations, so often found in papers of Marchand (52) and his collaborators (34), (35), (36), or in textbooks of clinical hematology (71), (72), (68), as to the formation of myeloid cells in myeloid metaplasia from "clasmatocytoid adventitial cells" or "endothelium," have not always been supported by convincing descriptions and illustrations. Of course, the theoretical possibility of the formation of blood cells from the resting wandering cells, from the histiocytes of the common connective tissue, always remains, but in such cases it appears to me much simpler and more natural to try to trace the new blood cells back to blood lymphocytes, which have emigrated into the tissue or are stagnating in the vessels. This is for instance the case in the formation of bone marrow in the rabbit's kidney after ligation of its blood vessels (58).

In the myeloid and lymphoid tissues the formation of new hemocytoblasts, large basophilic lymphocytes, from the fixed histiocytes, the reticular syncytium, can be demonstrated (fig. 1, *q*, *2*, *aa*, *2*), but it is also of no common occurrence. Downey and Weidenreich (22) have

given descriptions of this phenomenon in normal lymph nodes. In in-vitro cultures of lymphoid tissue the transformation of the reticular cells into large basophilic lymphocytes sometimes can be seen with striking clearness (63). In this case the basophilic protoplasm of the large lymphocytes may for a long time keep the peculiar pigment granules, characteristic for the protoplasm of the reticular cells, as an evidence of their origin.

Of all the cells of the circulating blood the monocytes (the large mononuclears and the transitionals of Ehrlich) have offered the greatest difficulties to the elucidation of their relationships and origin. Ehrlich's original idea, that their oldest forms, the so-called transitionals, are gradually becoming transformed into granulocytes in the circulating blood, had to be abandoned. It is firmly established that they have to be placed into the category of the non-granulated white blood corpuscles and that the only granular inclusions they occasionally possess are the common azurophilic granules. Weidenreich (93) refused to separate them from the rest of the agranulocytes, the common lymphocytes, and believed them to be merely a peculiar type of lymphocytes. Their close relationship to lymphocytes was advocated also by Maximow (60), (63) and Downey (18), (19), (20). However, it is true that in the normal adult circulating blood it is not always easy to find an uninterrupted series of transitional forms between them and the lymphocytes. They seem indeed to maintain in the circulating blood a more or less independent position.

Their origin has been explained in the most different ways. Being numerous in the red pulp of the spleen, they have been thought by Türk (92) to originate in the spleen and were accordingly named splenocytes. Jolly (38) traces them back to the sinuses of the lymph nodes. The adherents of the orthodox dualistic theory, as for instance Nägeli (68), believe them to belong to the myeloid series of elements and to originate directly from the myeloid indifferent stem cell, the so-called myeloblast, or from the hypothetic progenitor of the latter, the lymphoidocyte (71), (72).

The histological characters of the monocytes remind one very closely of the free histiocytes. The monocytes cannot be sharply differentiated from histiocytes. Both have an abundant, only very slightly basophilic protoplasm, occasionally containing vacuoles, and actively ameboid. The nucleus in both is occupying a more or less excentric position and has an oval or kidney shaped form, a sometimes folded membrane and a much more delicate intranuclear network, than in the

nucleus of the large lymphocytes. Both are displaying phagocytic activity and a part of the monocytes of the blood, though not all (70), are also able to store vital dyes.

In fact, we have seen wandering cells of the histiocytic and monocytic type arise together and keep close relationship from the earliest stages of ontogenesis. They first appear in the lumen of the yolk vessels and originate there from the endothelium (fig. 1, *h*, *3*, *4*) (59), (81), (82). Later they are found everywhere in the diffuse mesenchyme, arising from the indifferent fixed cells in the form of histioid wandering cells, or free histiocytes (*1*, *u*, *3*, *4*). Soon they appear also in the general circulation, together with the basophilic, lymphocytoid elements, the hemocytoblasts. Whereas in the youngest stages they originate everywhere directly from the common, indifferent, fixed mesenchyme cells, in the later stages, when the endothelium, and then the fibroblasts, separate from the rest of the mesenchyme, their number increases partly through independent proliferation (*y*, *3*) and partly through neoformation from the cell lineage of the fixed, resting histiocytes, which keeps for ever the full hematopoietic potencies unrestricted (*x*, *dd*, *3*).

A secure basis for the solution of the problem of the origin and the genetic relations of the monocytes in the adult organism was given by the discovery of free histiocytes in the circulating blood (42), (88).

In various experimental conditions, especially after intravenous injections of particulate matter or colloidal solutions of vital dyes, the whole reticulo-endothelial system, all the histiocytes in the body and chiefly in the abdominal organs and in the bone marrow are entering a phase of functional stimulation. Many of them hypertrophy, become free and are carried as large, vitally stained elements into the blood of the right heart. This seems to occur periodically, the cells being shed off into the veins in showers (88). Most of these cells, however, do not enter the general circulation and seem to be filtered off in the capillaries of the lungs. The findings of the new Italian hematological school (30), (26), (33) concerning the common occurrence of free histiocytes in the peripheral blood in leucemias, etc., have to be accepted with extreme caution. Ringoen (78) has recently shown that the dry smear method can easily produce artifacts, so that common lymphocytes and even myelocytes will look like histiocytes.

In normal conditions the histiocytes probably never enter the circulation; but they produce everywhere in the tissues, and especially in spleen, liver, bone marrow, lymph nodes and omentum, through

proliferation, monocytes, which become a part of the elements of the circulating blood (fig. 1, *3*, *4*).

Where the histiocytes are lining blood channels or occasionally are freely floating in the latter, as isolated elements—in the spleen and liver—the monocytes, resulting from their mitotic division, are directly swept away with the blood current. From the sinuses of the lymph nodes the monocytes, produced by the parietal reticular cells, may be carried into the lymph of the thoracic duct and thus enter the blood. Some authors, however, deny the presence of monocytes in the lymph (46). Finally, the monocytes, originating in the general mass of the lymphoid and myeloid tissue, in the lymph nodes, spleen, bone marrow, etc., have to find their way into the blood stream through the meshes of the reticulum, through the preformed openings in the wall of the blood vessels or through the continuous endothelial wall by means of active migration.

In the proliferation of the histiocytes, resulting in the formation of monocytes, some of the properties of the histiocytes may be temporarily lost or concealed, for instance, the capacity of storing vital dyes; but under suitable external conditions, as in inflammation, the monocytes, as we shall see, can again acquire the characters and functions of their mother cells, the histiocytes, and may again return to the quiescent condition of the fixed histiocytes.

As the fixed and even the free histiocytes are cells, belonging to the connective tissue, the monocytes of the blood, the offspring of the histiocytes, are to be considered as the most typical connecting link between blood and connective tissue, as the blood cells, standing nearest of all to the latter.

A part of the problem of the monocytes is the question of their genetic relations to the lymphocytes.

In the adult and especially the human organism the monocytes of the circulating blood, according to the dualistic theory (68), are sharply separated from the lymphocytes and belong to the myeloid system, being offspring of the so called myeloblasts. This point of view is based chiefly on minute differences in the nuclear structure as seen in dry blood smears and on the absence of the oxydase reaction in the lymphocytes. Kiyono (42) also draws a sharp line between the histiocytes and monocytes on the one, and the lymphocytes on the other hand. But contrary to the dualists, he declares the former as independent from the myeloid system as well, and thus seems to have a kind of triadic ("trialistisch" in German) conception of hematopoiesis,

the lymphocytes, the histiocytes together with the monocytes and, finally, the granulocytes together with the erythrocytes representing in his opinion three distinct independent cell groups. The chief proof for the sharp distinction between the histio-monocytes and the lymphocytes is for him the capacity of the former to store vital dyes, as for instance lithium-carmine or trypanblue—a phenomenon never observed in the lymphocytes.

The school of Marchand, for instance Herzog (35), (36), is also inclined to sharply differentiate between lymphocytes and histio-monocytes.

The unitarian theory of hematopoiesis never hesitated to bring the histiocytes and monocytes in close connection with lymphocytes. In the early embryo, as we have seen, both arise practically at the same time in the lumen of the yolk vessels (fig. 1, *g*, *2*, *h*, *3*, *4*) and in the general mesenchyme as well (*k*, *t*, *2*, *l*, *u*, *3*, *4*), and in the later stages of the embryonic development everywhere transitional forms between them can be seen, especially in the primordia of the lymph nodes (*i*, *m*, *v*). As I have already mentioned, the circulating embryonic blood also contains from the very beginning basophilic hemocytoblasts as well as histioid wandering cells, i.e., histiocytes or monocytes. The opinion of the italian authors (di Guiglelmo (33) and others) as to the exclusive presence in the embryonic blood of histiocytes is based on defective technique.

It is certainly true, that in the normal circulating blood of the adult transitional forms between the two cell types are not always present and that the lymphocytes in their usual condition do not store vital dyes. But the occasional absence of transitional forms in the blood does not mean their absence in the blood forming organs or, for instance, in the omentum. As regards the different behavior toward vital dyes, this criterion cannot be considered as an infallible and absolute indicator of cell origin and cell relationship (19), (20). Storage of vital dyes is the symptom of a certain functional activity of the cell and can undergo profound changes according to the various phases of functional conditions. In fact, we shall see below that in inflammation small or medium sized lymphocytes, absolutely refusing vital dyes, may quickly become transformed into polyblasts, storing large quantities of dyes. The same can be easily observed in the living condition in in-vitro cultures of lymphoid tissue, prepared with a nutritive medium, containing a vital dye, for instance, lithium carmine (63).

Downey (18) has shown by a simple and convincing experiment that the vital stain is not a sufficiently sensitive and reliable reagent for differentiating cellular types. If lithium carmine be injected into a section of a vein, filled with blood and isolated by means of two ligatures, the lymphocytes hypertrophy and accumulate carmine granules. Moreover, it has to be pointed out that Kiyono (42), as well as Herzog (34), in many places of their papers have themselves admitted that a transformation of small lymphocytes into vitally stainable polyblasts or histiocytes "sometimes" or "to a certain extent" still occurs. Such a concession evidently completely invalidates the whole theory of the independence of both cell types from each other.

The conclusion therefore is that in the adult organism the lymphocytes, on the one hand, through individual transformation, usually not accompanied by mitosis, may (inflammation, see below) produce cells of histiocytic and monocytic type (polyblasts) (fig. 1, *kk*, *ll*); on the other hand, the histiocytes, under certain conditions, for instance, as mentioned above, in tissue cultures, may become transformed into lymphocytes. A mitosis of a free histiocyte might also, perhaps, give rise to a pair of lymphocytes (*ff*).

In the normal adult organism, where the vital wear and tear of the elements is relatively insignificant and the toll is strictly balanced by the regenerative processes, the relations between blood and connective tissue and especially the functions and the prospective potencies of the histiocytes are not always distinct. They clearly manifest themselves during inflammation and in tissue cultivated outside the body.

The inflammation can be considered from the viewpoint of comparative pathology as an abnormal increase of a multitude of interrelated functions of various mesodermic derivatives (79). These functions have to free the connective tissue of the respective area of foreign substances and manifest themselves in inflammatory extra- or intracellular parenteral digestive processes. As in the normal organism occasional local chemical changes in the surroundings of tissue cells also occur, with a corresponding reaction of the latter, it is quite possible to admit the existence of "physiological inflammations," differing from the true inflammation only quantitatively. After explantation into a nutritive medium outside the body the cells of the explant also find themselves surrounded by new, mostly foreign substances and, although in this case the regulatory influence of the whole organism, the circulation and the nervous impulses are lacking, the elements still behave partly in the same way, as in inflammation.

For a clear understanding of the inflammatory reaction a thorough knowledge of its histology is necessary. It was established long ago that in inflammation elements of the local connective tissue and of the blood as well are taking part. Unfortunately, in the papers on the general pathology of inflammation one often finds very confused ideas of the morphological side of the problem (79).

In inflammation, besides the emigration of special granular leukocytes and the proliferation of fibroblasts, gradually forming the chief mass of the granulation or scar tissue, a most important rôle is played by the so-called polyblasts—mononuclear, ameboid, phagocytic elements, in their structure closely resembling monocytes and motile histiocytes. The question of their origin, which was so long debated, seems now to be definitely settled (55). They arise partly through direct transformation of the local wandering cells, the free histiocytes of the tissue (*hh*), and through mobilization and isolation of the local fixed histiocytes or resting wandering cells (*ii*). But another, and, usually, larger part of the polyblasts originates from the blood lymphocytes (*kk*, *ll*) and monocytes (*mm*), emigrating out of the blood vessels and undergoing a remarkably swift hypertrophy on the field of inflammation. These emigrated cells very soon become indistinguishable from the polyblasts of local origin. This startling fact of an apparently dual origin of a cell group is quite natural—we have seen that the histiocytes in the embryo are partly formed by lymphoid or histioid wandering cells, returning to the quiescent condition (*ee*) and histiocytes as well as lymphocytes and monocytes have the same prospective potencies. The true endothelium (see below) seems not to play an important rôle.

If inflammation occurs in blood forming tissue, for instance in lymph nodes, the polyblasts arise chiefly from the reticular cells, the local histiocytes; but later on, the local lymphocytes may also undergo the same transformation (3).

The polyblasts may form epithelioid or giant cells or stay in the newly formed connective tissue as wandering cells, as free histiocytes (*nn*); in the late stages, during scar formation, however, the majority of them becomes transformed into flat, spindle shaped or branched resting polyblasts, which correspond in every respect to the fixed histiocytes or resting wandering cells of the normal loose connective tissue (*oo*). If a new inflammation begins, they again awake and again become ameboid, active, phagocytic polyblasts.

The endeavors of many investigators (especially Marchand (52), (53) and his students, for instance, Herzog (34), (35) and others), to shatter the idea of the participation of hematogenous, emigrated lymphocytes and monocytes in the production of stable elements of the inflammatory granulation tissue, have been unsuccessful. They tried to trace back all the polyblasts to the so-called adventitial cells of Marchand, mentioned above, i.e., to histiocytes, or to endothelium. The first, most important fact against this presumption is, that the tissue in acute inflammation is overflooded in the shortest time, during the first hours by innumerable quantities of small polyblasts, looking exactly like small or medium sized lymphocytes. No proliferation can be made responsible for this cell production, as there are no mitoses to be found at this time. The idea of a rapid amitotic division of local elements, expressed by some investigators, is absurd. On the other hand a careful study of the vessels in the inflamed area in the early stages reveals numerous pictures of emigrating lymphocytes and monocytes. Furthermore, all stages of the transformation of freshly emigrated blood lymphocytes into large, phagocytic, ameboid cells can be easily observed in the tissue. In vitro cultures give us the opportunity of doing this directly in the living condition (63).

The foci of infiltration with small round cells, so commonly met with in all cases of chronic inflammation in various parts of the organism are probably always originating through emigration of hematogenous small lymphocytes (86).

In tuberculosis the polyblasts of the same double origin are playing the chief rôle in the formation of the tubercle. If tubercles are formed in lymphoid tissue, the epithelioid and, implicitly, the giant cells arise partly from the mobilized ameboid reticular cells, partly from lymphocytes of local origin, hypertrophying and joining in their further development the reticular cells (65).

It is quite certain that under normal conditions the common round wandering cells of the loose connective tissue partly also originate from emigrated blood monocytes (*pp*) and lymphocytes (*qq*). Transitional forms between these blood elements and the larger types of wandering cells, the free histiocytes of the loose connective tissue, are easily found everywhere, especially in the omentum.

As regards the plasma cells, they may be looked upon as a special type of polyblasts occurring under normal as well as pathological (inflammatory) conditions. However, they apparently never arise from local histiocytes, but always directly from emigrated (small)

blood lymphocytes or from the lymphocytes of the hematopoietic organs (*rr*). Theoretically it is quite possible that they may also arise from lymphocytes of local histiocytic origin.

In in-vitro cultures of various types of connective tissue we see practically the same cell transformation taking place, as in inflammation—with the exception, of course, of the emigration of blood elements. The fibroblasts expand and proliferate and form the foundation of the new tissue. The histiocytes become motile, ameboid, phagocytic elements—polyblasts. The lymphocytes, if present, also may show polyblastic transformation. But in cultures of lymphoid tissue another very important process may be observed. In the lymph nodes of the adult organism, even in inflammation, as I mentioned, new lymphocytes are rarely seen to arise anew from the histiocytes, the reticular cells. The independent proliferation of the present lymphocytes seems to be sufficient. In tissue cultures of lymphoid tissue, on the contrary, it is easy to observe neoformation of typical large basophilic lymphocytes from the reticular cells (*aa*, *2*).

Very often cells with eosinophilic granules are found in the connective tissue, especially in the rat, the mouse and the guinea pig, where they seem to be a normal cellular constituent of the diffuse loose connective tissue of the adult (56). They certainly have to be distinguished from the scarce eosinophilic myelocytes and granulocytes, arising in the diffuse mesenchyme of the young embryo and disappearing in the later embryonic period. In all mammals they occur regularly in the tunica propria of the intestinal mucous membrane (83), (94). The physiological meaning of this so-called local or tissue-eosinophilia is unknown and even the origin of these eosinophiles of the connective tissue is an apparently unsettled question (87).

In the common loose connective tissue of the rat, the mouse and the guinea pig the eosinophiles always appear relatively late, gradually increasing in number during the first weeks of extra-embryonic life (1). No proofs of their local origin can be found and they are to be considered as true eosinophilic leukocytes, emigrated out of the blood vessels and staying in the connective tissue for the sake of some unknown function, perhaps connected with intestinal parasitic infections. The same hematogenous explanation can be applied probably to most of the other cases of tissue eosinophilia.

In the intestinal wall the conditions are not so simple. As far as the eosinophiles with a polymorphous, bilobed nucleus are concerned, they certainly, also might have come as mature eosinophile leukocytes

from the blood vessels. But many authors claim that eosinophilic myelocytes with a compact, round or oval nucleus and even with mitoses, are also found in the tunica propria from early stages of embryonic development. Thus, at least a part of the eosinophiles of the intestine might originate in loco, presumably from lymphocytes.

The eosinophile cells, in other places, for instance in the omentum, described by some authors (14), (69) are possibly no true eosinophile leukocytes nor myelocytes at all, but lymphoid cells, elaborating in their protoplasm a peculiar acidophilic substance in the form of large granules or lumps of uneven size and shape. They might be identical with similar cells, described by Weill (94) as "Schollenleukozyten" in the intestinal mucosa, or with plasma cells, elaborating acidophilic inclusions (24) or with lymphoid cells with acidophilic granules, found by Solucha (89) in the loose connective tissue of the adult chick, etc.

A special mention has to be made of the omentum. Here the tissue shows a structure extremely variable in normal conditions and normally very often gives the impression of inflammation ("physiological inflammation" of Rössle). It contains, besides fibroblasts, especially in the milky spots, enormous quantities of histiocytes in resting and in active, ameboid condition. Great quantities of the free ameboid histiocytes are constantly shed off into the peritoneal cavity and are floating there as large, free, phagocytic elements, which I call exudate polyblasts. Besides, the omentum always contains some large lymphocytes and a various, sometimes enormous, amount of small lymphocytes and of plasma cells, derived from lymphocytes. The idea of the local production of lymphocytes from fixed connective tissue cells (histiocytes) has been always advocated especially for the omentum, by Pappenheim, Marchand (52) and others. It is possible that a certain quantity of lymphocytes, especially the large ones, originates in this way (*dd*, *3*, *2*). But the great majority of the lymphocytes in the omentum are present and multiply as such in loco from the time of the embryonic life. Besides, Wjereszinski (95) has recently shown that the blood vessels of this organ always contain a very large amount of emigrating lymphocytes. In this respect the omentum is again analogous to inflamed tissue. Another similarity is the transformation of the local or emigrated small lymphocytes into free histiocytes and into exudate polyblasts, easily observed in most of the cases.

As regards the mesothelium, the squamous cells, covering the serous membranes, their relations to the connective tissue and blood are as yet not quite clear. The idea that they are able to produce through

proliferation on the one hand fibroblasts, on the other exudate polyblasts and even lymphocytes (85), (15), (38), has not been confirmed. Lippman and Plesh (49) attempted to give this idea a conclusive proof by provoking inflammation in the pleural cavity of animals, whose leukocytes in the circulating blood had been previously destroyed by injections of Thorium X. They saw typical exudate polyblasts and lymphocytes appear in the cavity and, as there were no lymphocytes in the blood, explained their origin through proliferation of the mesothelium. It is clear that this conclusion is by no means convincing, as it has never been shown that the Thorium X should destroy all the local histiocytes and lymphocytes present in the tissue of the serous membrane itself.

Some authors believed that the mesothelial cells are simply modified fibroblasts and that they can be formed by the latter under experimental conditions (7). W. Lewis (47) saw the fibroblasts in tissue cultures assume a squamous shape and a general structure, typical for mesothelium. Wjereszinski (95) has shown that some of the free cells of the peritoneal exudate, if explanted, give rise to colonies of fibroblasts; he does not however identify them directly with mesothelial elements. Other investigators point out that the mesothelial elements differ from common fibroblasts and display some qualities of true epithelium. In my recent (unpublished) experiments with explantation of mesothelium its cells behaved differently from true fibroblasts. Evans and Scott (28) and Cunningham (9), (10), (11) also sharply differentiate the mesothelium of the omentum from the fibroblasts.

Evidently new investigations are required for solving this problem.

Typical fibroblasts, at least in the common loose connective tissue, never produce wandering cells or lymphocytes; they have lost the respective potencies. But there remains the question about the inverse process—the transformation of lymphocytes and wandering cells of histiocytic type into fibroblasts. The French authors have always accepted this possibility as something quite natural (77), (23), (15). But, speaking generally, this idea nowadays seems to be very unpopular (52), (90). Yet, I suppose, there are facts speaking in its favor. In 1902 (55), following the changes undergone by the polyblasts on the field of inflammation, I saw these cells first acquire a resting, fixed position, as described above, and finally, during scar formation, gradually lose their typical histological character—the dark irregular nucleus and the sharp outlines of the protoplasm—and acquire instead, structural characters, typical for the fibroblasts. Thus some, at least, of the

resting polyblasts, partly representing former lymphocytes, apparently become transformed into fibroblasts and may take part in the production of collagen. In 1914 Awrorow and Timofejewski (2), cultivating human leucemic blood in rabbit plasma, succeeded in obtaining cultures of spindle-shaped, fibroblast-like cells from the white blood corpuscles. Recently Carrel and Ebeling (6) confirmed their results; they obtained in vitro, from explanted white blood corpuscles of the blood of the adult chick, probably the monocytes, typical cultures of fibroblasts. Of course, production of collagen by these spindle shaped cells, originating from white blood corpuscles, has not been shown. The cells, however, were exactly similar to the common fibroblasts, which can be cultivated from connective tissue and which also, during many years of in-vitro existence in Carrel's laboratory, have not been seen to produce collagen. We have to conclude, therefore, that a transformation of fixed and free histiocytes and of certain types of white blood corpuscles—the lymphocytes and monocytes, which cannot be sharply separated from each other—into common connective tissue cells, fibroblasts, is possible (fig. 1, *ss*, *tt*, *uu*). At any rate, there are no facts directly contradicting this hypothesis. Similar ideas have been recently expressed by Benninghoff (4), who finds in various types of connective tissue numerous transitional forms between the wandering and the fixed elements.

Finally I have some words to add concerning the endothelial problem. It is a widespread belief that endothelium is capable of producing blood elements and in inflammation, wandering cells, phagocytes, epithelioid cells, giant cells, etc. (31). Mallory (50) calls, for instance, the monocytes "endothelial leukocytes." Patella (73) has published a long series of papers trying to give convincing proofs for the endothelial origin of monocytes and lymphocytes. McJunkin (41) also believes that the common endothelial cells are able to produce monocytes through proliferation and isolation. As a striking example of the one-sided influence of this dominating idea, I may refer to the recent articles of Herzog (36), who ascribes to the endothelium an exclusive and practically omnipotent capacity of producing everything, beginning with smooth muscle cells and fat cells and ending with lymphocytes and other blood cells and even all kinds of tumor cells. Marchand in his latest paper (53) also expresses a similar opinion.

In my studies on embryonic histogenesis, on inflammation and on in-vitro life of the mammalian tissues, I was unable to convince myself of the truth of this idea.

It is quite possible that in the lower vertebrates the general endothelium remains in a more or less undifferentiated condition, keeps a part of its embryonic prospective potencies and, accordingly, plays a more prominent hematopoietic rôle. In axolotl and triton larvae I have seen production of lymphocytes and even erythroblasts on a large scale by the endothelium in certain parts of the vascular system. Jordan (39) has found the same in the frog and the reptiles. But in the mammals it is different. Here the endothelium of the common blood and lymph vessels seems to be a highly specialized tissue with restricted potencies of development.

As we have seen, during a certain, very short time after the dissolution of the blood islands, the endothelium of the yolk vessels furnishes a small additional quantity first of primitive blood cells (*d*) and then of hemocytoblasts (*g*, *2*), free histiocytes and monocytes (*h*, *3*, *4*). Furthermore, as I have found (59) and Emmel (25) and Jordan (40) have confirmed, the endothelium in certain restricted parts of the embryonic vascular system, namely, in the ventral wall of the caudal aorta, sheds off clusters of proliferating histio-monocytes and lymphoid hemocytoblasts even in slightly older embryos (rabbit embryos 10 to 10½ days) (*vv*, *2*). These clusters cannot be considered as retarded blood islands; they do not consist of primitive blood cells, but of histioid and lymphoid wandering cells, i.e., histiocytes and hemocytoblasts; the latter produce through differentiating proliferation secondary erythroblasts, which are swept away by the blood current. Afterwards, and not only in the adult, but also in the later stages of embryonic life, I never was able to find any proof for the hematopoietic activity of true endothelium (*z*).

It has been shown (44) that in certain cases, after direct lesions of the vascular walls, common endothelial cells, sometimes in large sheets, can be found in the blood. But these elements have no resemblance with the so-called endothelial leukocytes, i.e., monocytes.

During inflammation, simple, purulent or specific, for instance tuberculous, the common endothelium also never produces ameboid, wandering cells, polyblasts, epithelioid or giant cells. The same is true for tissue cultures, where the transformations of the different cell types can be followed with extraordinary clearness. The endothelium of the blood vessels in inflammation and in vitro as well, either remains as it was and forms in the usual way new capillary sprouts; or the connections between its cells become loose, the cells separate from each other, and the result is the transformation of endothelium into fibro-

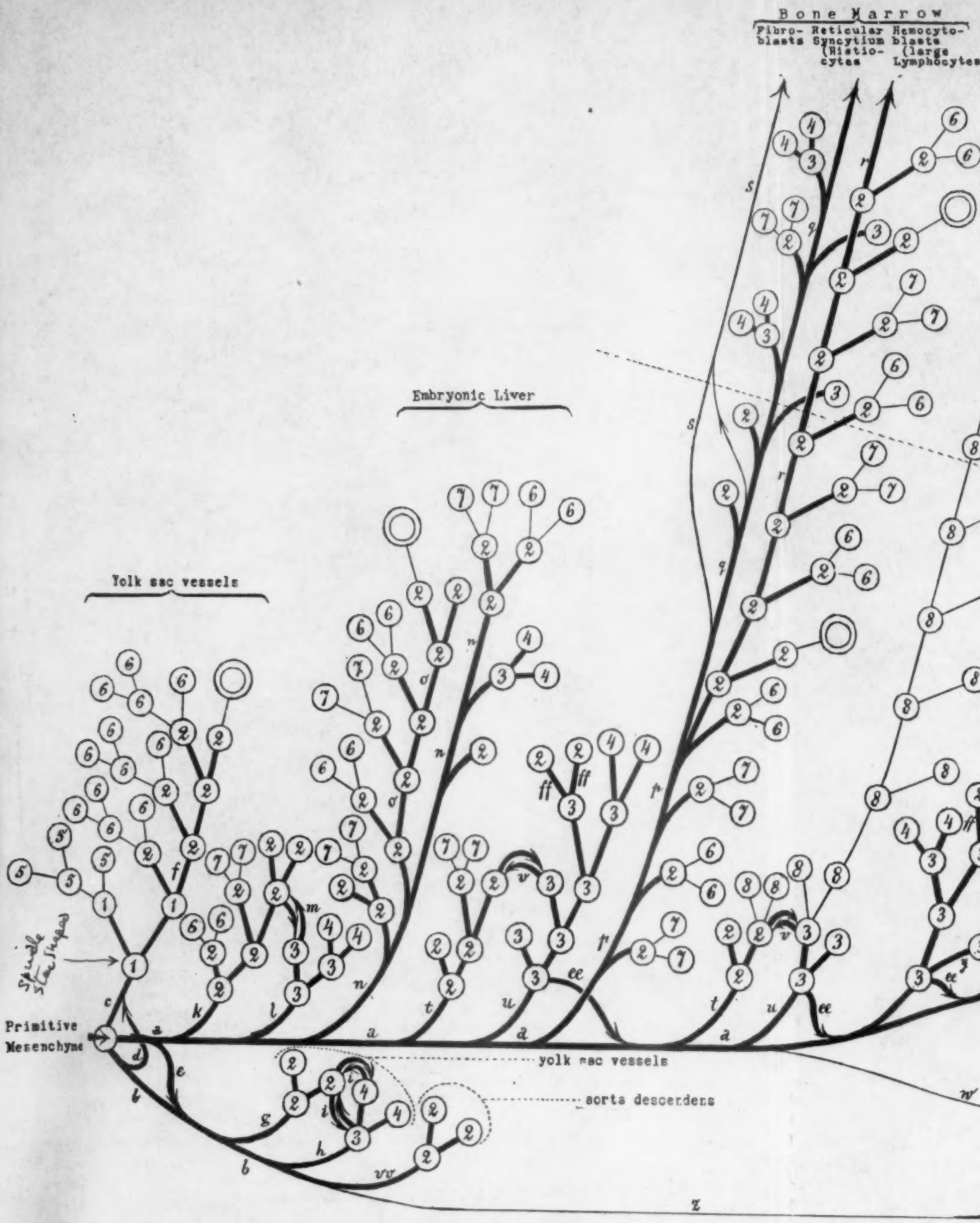
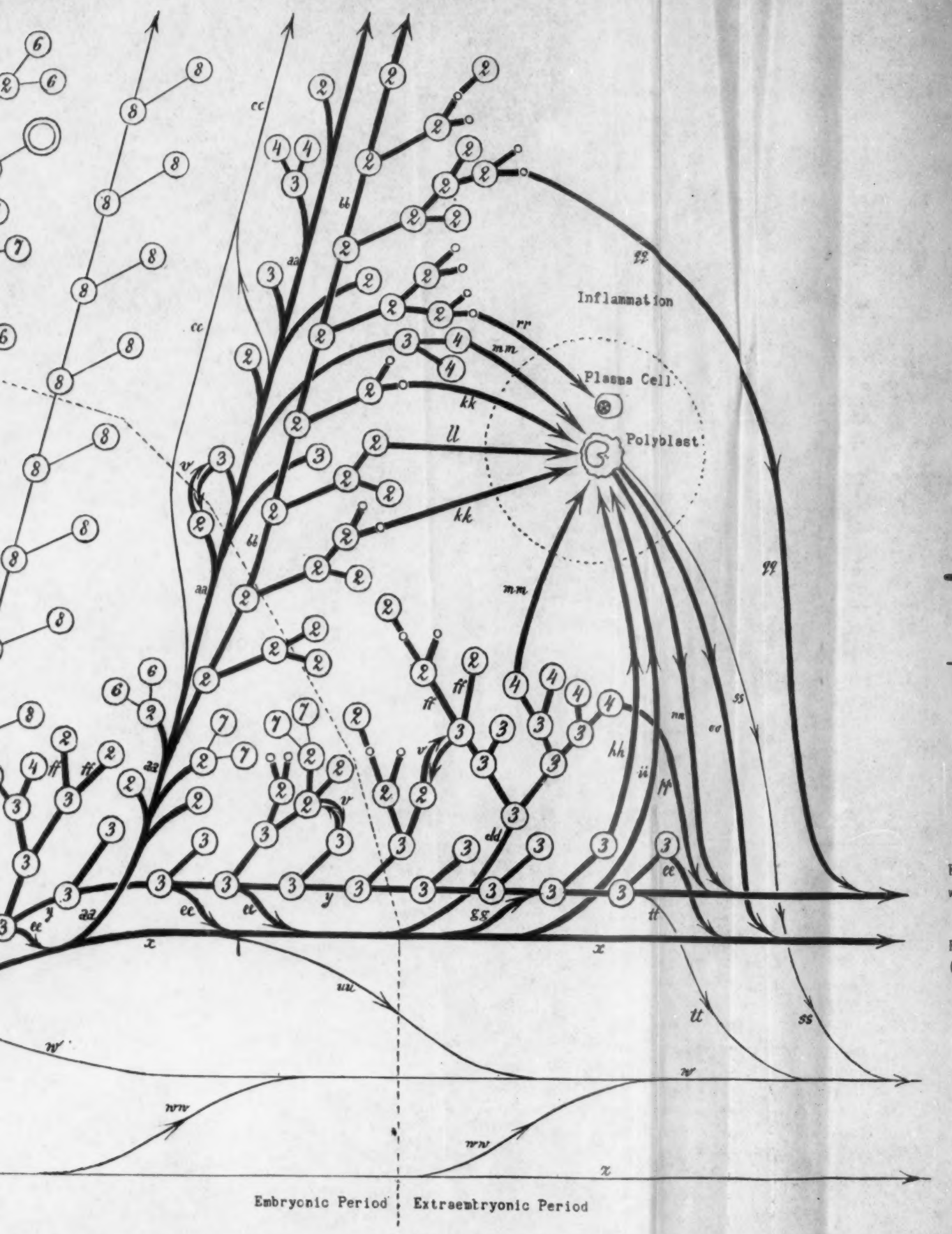
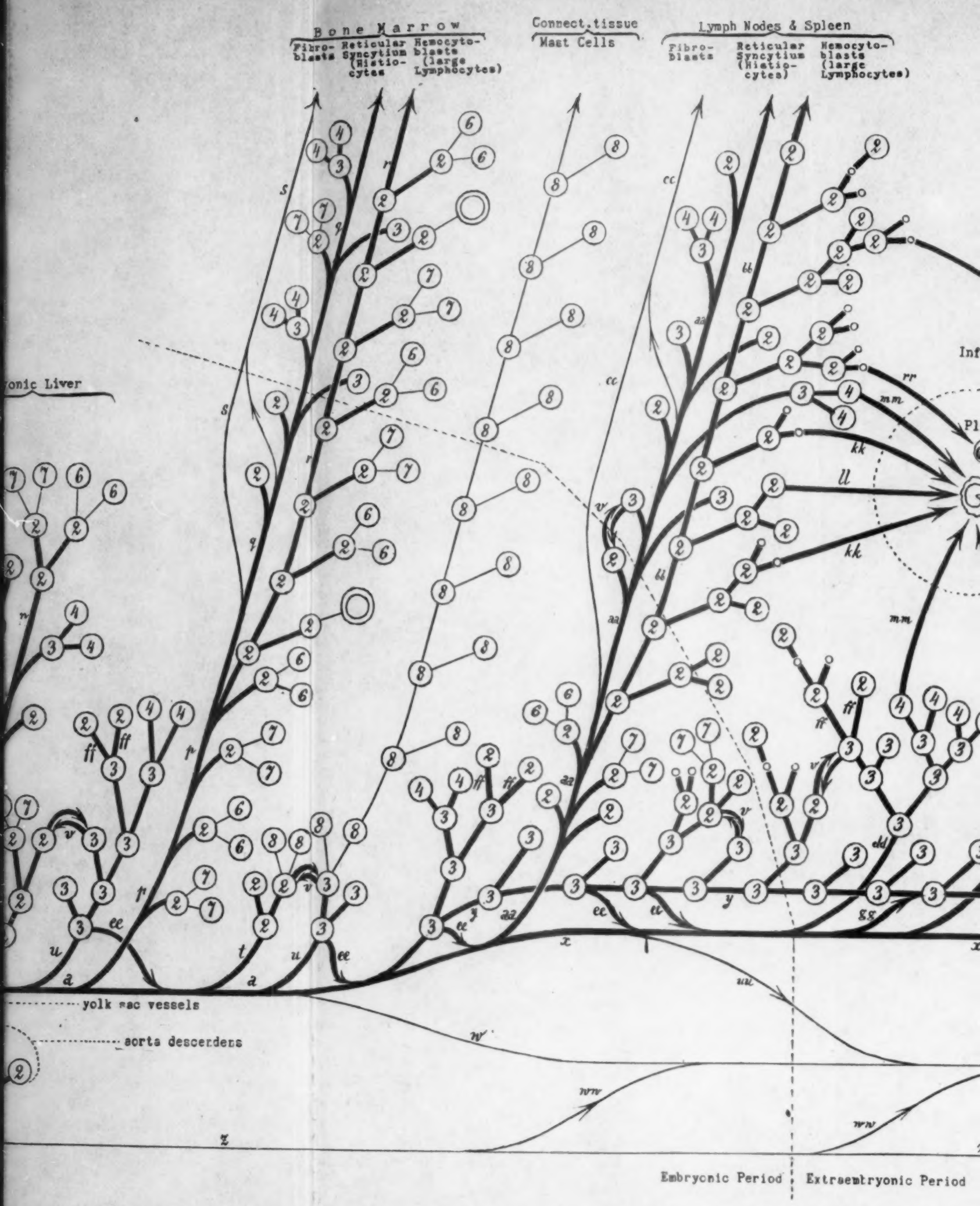


Fig. 1. The diagram represents the development and the interrelations of the blood- and connective tissue-cells of the mammals in the embryonic and extraembryonic life. The numbered circles represent the various cell types, the different cell lineages are shown by lines and marked either with letters, placed at the side, or with legends, placed at the end of the lines. The heavy lines are cell lineages, in which only mitotic division and structural changes, but no differentiation of the cells occurs and the hematopoietic potencies remain unrestricted. From these lines in many places isolated, free and motile elements split off, without cell division and merely through direct cell transformation. The light lines are cell lineages, where during the differentiating proliferation or individual transformation the original amount of embryonic hematopoietic potencies decreases and special cell types are produced.

a, common fixed mesenchyme cells; *b*, primitive endothelium; *c*, primitive blood cells; *d*, additional formation of primitive blood cells by the endothelium of the yolk sac vessels; *e*, additional formation of endothelium by the mesenchyme in the area pellucida and the body of the embryo; *f*, cell lineage of the hemocytoblasts (large lymphocytes) in the yolk sac vessels; *g*, additional formation of hemocytoblasts by the endothelium of the yolk sac vessels; *h*, formation of free histiocytes ("endothelial phagocytes") by the endothelium of the yolk sac vessels; *i*, transitional forms between hemocytoblasts and free histiocytes and monocytes (endothelial phagocytes) in the yolk sac vessels; *k*, hemocytoblasts, arising extravascularly from the common fixed mesenchyme cells in early embryonic stages; *l*, histioid wandering cells, arising between the hemocytob lineage of fixed mesench embryonic liver, produci in the embryonic liver marrow, resorbing the c *q*, the fixed histiocytes hemocytoblasts (large l (and fat cells?) of the cells in the middle of th from fixed mesenchyme tween the hemocytobla common diffuse connect cells) of the common di wandering cells) of the *aa*, cell lineage of fixed of free, independently fibroblasts in the lymp histiocytes in the comm into fixed histiocytes (



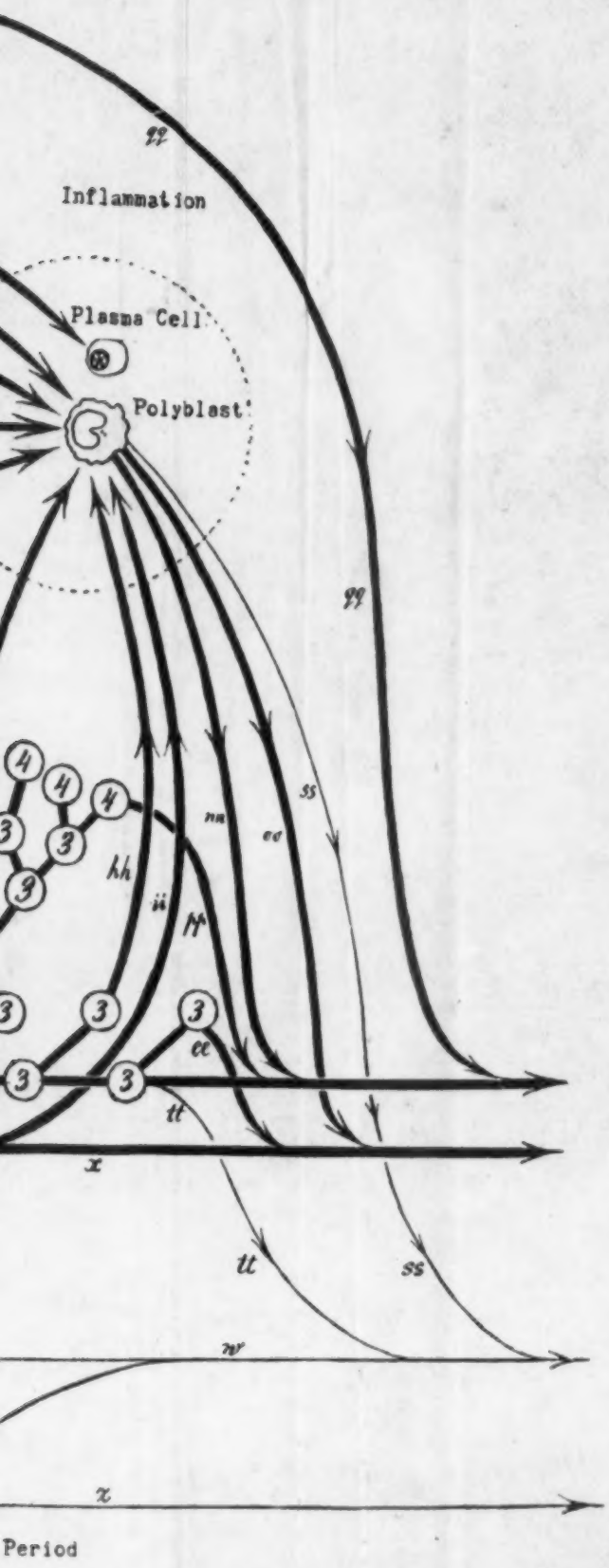
, arising from the common fixed mesenchyme cells; *m*, transitional forms
mocytoblasts and the histioid wandering cells in the mesenchyme; *n*, cell
mesenchyme cells (partly lining the blood sinusoids as endothelium) in the
, producing hemocytoblasts; *o*, cell lineage of free proliferating hemocytoblasts
ic liver; *p*, cell lineage of fixed mesenchyme cells in the embryonic bone
ng the cartilage and forming hemocytoblasts; it splits into three cell lineages;
tiocytes (reticular syncytium) of the bone marrow; *r*, the free proliferating
(large lymphocytes, myeloblasts) of the bone marrow, and *s*, the fibroblasts
of the bone marrow; *t*, hemocytoblasts, arising from the fixed mesenchyme
dle of the embryonic life; *u*, free histiocytes (histioid wandering cells) arising
enchyme cells in the middle of the embryonic life; *v*, transitional forms be-
ocytoblasts and free histiocytes; *w*, cell lineage of the fibroblasts of the
connective tissue; *x*, cell lineage of the fixed histiocytes (resting wandering
mmon diffuse connective tissue; *y*, cell lineage of the free histiocytes (histioid
) of the common diffuse connective tissue; *z*, endothelium of blood vessels;
of fixed syncytial histiocytic elements in the lymph nodes; *bb*, cell lineage
ndently proliferating hemocytoblasts in the lymph nodes; *cc*, cell lineage of
he lymph nodes; *dd*, free histiocytes, arising through mobilization of fixed
he common diffuse connective tissue; *ee*, transformation of free histiocytes
ocytes (resting wandering cells); *ff*, origin of a pair of hemocytoblasts from
a mitosis of a free histiocyte; *gg*, mobilizati
in the common diffuse connective tissue and
transformation of free histiocytes (local "h
into polyblasts in inflammation; *ii*, mobili
cells) and their transformation into polyb
emigrated small lymphocytes into polybl
(hemocytoblasts) into polyblasts; *mm*, tran
nn, transformation of polyblasts in the infl
newly formed connective tissue, free histiocy
polyblasts (fixed histiocytes) during scar f
monocytes into common wandering cells (f
qq, transformation of emigrated blood ly
histiocytes) of the normal connective tissu
plasma cells; *ss*, transformation of polybl
into fibroblasts; *tt*, transformation of free
of fixed histiocytes into fibroblasts; *vv*, form
the ventral wall of the caudal aorta in y
common endothelium into fibroblasts.



d the interrelations of the blood- and
yonic and extraembryonic life. The
e different cell lineages are shown by
le, or with legends, placed at the end
h only mitotic division and structural
d the hematopoietic potencies remain
d, free and motile elements split off,
ansformation. The light lines are cell
ion or individual transformation the
es decreases and special cell types are

ndothelium; *c*, primitive blood cells;
endothelium of the yolk sac vessels;
achyme in the area pellucida and the
lasts (large lymphocytes) in the yolk
by the endothelium of the yolk sac
phagocytes") by the endothelium of
mocytoblasts and free histiocytes and
vessels; *k*, hemocytoblasts, arising
ls in early embryonic stages; *l*, histioid

wandering cells, arising from the common fixed mesenchyme cells; *m*, transitional fo
between the hemocytoblasts and the histioid wandering cells in the mesenchyme; *n*,
lineage of fixed mesenchyme cells (partly lining the blood sinusoids as endothelium) in
embryonic liver, producing hemocytoblasts; *o*, cell lineage of free proliferating hemocytobl
in the embryonic liver; *p*, cell lineage of fixed mesenchyme cells in the embryonic b
marrow, resorbing the cartilage and forming hemocytoblasts; it splits into three cell linea
q, the fixed histiocytes (reticular syncytium) of the bone marrow; *r*, the free proliferat
hemocytoblasts (large lymphocytes, myeloblasts) of the bone marrow, and *s*, the fibrobl
(and fat cells?) of the bone marrow; *t*, hemocytoblasts, arising from the fixed mesenchy
cells in the middle of the embryonic life; *u*, free histiocytes (histioid wandering cells) ari
from fixed mesenchyme cells in the middle of the embryonic life; *v*, transitional forms
tween the hemocytoblasts and free histiocytes; *w*, cell lineage of the fibroblasts of
common diffuse connective tissue; *x*, cell lineage of the fixed histiocytes (resting wande
cells) of the common diffuse connective tissue; *y*, cell lineage of the free histiocytes (hist
wandering cells) of the common diffuse connective tissue; *z*, endothelium of blood ves
aa, cell lineage of fixed syncytial histiocytic elements in the lymph nodes; *bb*, cell lin
of free, independently proliferating hemocytoblasts in the lymph nodes; *cc*, cell lineag
fibroblasts in the lymph nodes; *dd*, free histiocytes, arising through mobilization of
histiocytes in the common diffuse connective tissue; *ee*, transformation of free histioc
into fixed histiocytes (resting wandering cells); *ff*, origin of a pair of hemocytoblasts



① Primitive Blood Cells

② Hemocytoblasts
(Large Lymphocytes, Lymphoid Wandering Cells)

③ Free Histiocytes
(Histioid or histiogenous Wandering Cells)

○ Small Lymphocytes

④ Monocytes

◎ Megakaryocytes

⑤ Primary Erythroblasts and Erythrocytes

⑥ Secondary Erythroblasts and Erythrocytes

⑦ Granulocytes

⑧ Connect. tissue Mast Cells

——→ Lines of development with potencies unrestricted

——→ Lines of development with potencies restricted

Free (histioid or histiogenous wandering Cells of Connect. Tissue
Free Histiocytes

Fixed Histiocytes
(Festing Wandering Cells)

Fibroblasts

Endothelium

} Common loose Connective Tissue and Omentum

ional forms
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ium) in the
ocytoblasts
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ell lineages;
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a mitosis of a free histiocyte; *gg*, mobilization of fixed histiocytes (resting wandering cells) in the common diffuse connective tissue and their transformation into free histiocytes; *hh*, transformation of free histiocytes (local "histioid" wandering cells of connective tissue) into polyblasts in inflammation; *ii*, mobilization of fixed histiocytes (resting wandering cells) and their transformation into polyblasts in inflammation; *kk*, transformation of emigrated small lymphocytes into polyblasts; *ll*, transformation of large lymphocytes (hemocytoblasts) into polyblasts; *mm*, transformation of blood monocytes into polyblasts; *nn*, transformation of polyblasts in the inflamed area into common wandering cells of the newly formed connective tissue, free histiocytes; *oo*, transformation of polyblasts into resting polyblasts (fixed histiocytes) during scar formation; *pp*, transformation of emigrated blood monocytes into common wandering cells (free histiocytes) of the normal connective tissue; *qq*, transformation of emigrated blood lymphocytes into common wandering cells (free histiocytes) of the normal connective tissue; *rr*, transformation of small lymphocytes into plasma cells; *ss*, transformation of polyblasts (partly former lymphocytes or monocytes) into fibroblasts; *tt*, transformation of free histiocytes into fibroblasts; *uu*, transformation of fixed histiocytes into fibroblasts; *vv*, formation of hemocytoblasts by the endothelium of the ventral wall of the caudal aorta in young embryonic stages; *ww*, transformation of common endothelium into fibroblasts.

blasts (*ww*). The development in the opposite direction, from fibroblasts to endothelium, on the contrary, cannot be observed. This is also the opinion of Marchand (52).

There were many authors who believed having seen neoformation of endothelium and blood vessels from connective tissue cells (for instance Minervini (66)), but their conclusions never could be confirmed.

In connection with the discussion of the rôle played by the endothelium in hematopoiesis and in inflammation, the so-called vasoformative cells of Ranvier (75) may be mentioned. They have now only historical interest. Ranvier described them in the omentum of new born animals as large, elongated or round, sometimes branched, elements, containing in their protoplasm red and white blood corpuscles. They were supposed to be connective tissue cells, producing new vessels and, at the same time, through "endocellular" formation, blood elements. Our present knowledge of the fundamental hematological phenomena is incompatible with such an assumption. Several investigators (Martinoff (54) and others) have conclusively shown that the "cellules vasoformatives" of Ranvier have nothing to do with the so called angioblasts and with hematopoiesis in general. They are merely parts of growing capillaries, which become detached from the general capillary network and are undergoing involution. The presence of erythrocytes and leukocytes in their interior does not indicate hematopoiesis; on the contrary, these elements here often show signs of degeneration.

The unsettled condition of the endothelial problem seems to be due simply to a different interpretation of the term "endothelium" by the various authors.

On the one hand, in the adult mammal no evidence can be found of the true endothelium of the blood- and lymph- vessels and the heart being able to play an important rôle in hematopoiesis or producing wandering, phagocytizing cells in inflammation. Of all the cells of the connective tissue the endothelium seems to keep a close relationship only with the fibroblasts.

On the other hand it is a well established fact that in some organs—the spleen, the liver, the lymph nodes, the bone marrow—blood or lymph channels are partly lined with flattened histiocytes, which are really able not only to become detached from the wall and to change into motile phagocytic elements, the macrophages, but also to produce, through proliferation, monocytes, and, under special conditions, large lymphocytes, i.e., hemocytoblasts and all other blood cells.

Most of the investigators, who believe to have proved the hematopoietic or the inflammatory activity of endothelium, in reality have had to do with these histiocytes. If the latter are called endothelium, then, certainly, the idea of the hematopoietic activity of endothelium might seem to be well established. But the name endothelium has been created for the designation of the flat cells lining the walls of all common blood and lymph vessels and the heart and for these elements we have no other name. The histiocytes cannot be called endothelium, because in the adult mammal they are totally different and independent from the true endothelium as well as from the fibroblasts. Moreover, their position in the wall of blood or lymph channels is not typical for them at all—the same elements are found in the reticulum of the blood forming tissues as reticular cells and are scattered in the omentum and in the common loose connective tissue as resting wandering cells.

The histiocytes the reticulo-endothelial elements, have to be distinguished from the common endothelium and further investigations will have to establish their distribution and localization in the various sections of the vascular system.

One of the most important achievements of modern hematology is the elucidation of the rôle of the histiocytes in the production of monocytes and of other blood cells. The histiocytes display a remarkable combination of special functional activities on the one, and of polyvalent embryonic potencies on the other hand. Due to their properties as nephrophagocytes, they are an important factor in the general metabolism, in internal secretion, in the elaboration of antibodies, etc. They share with the common endothelium (8) the capacity of elaborating fibrillar intercellular substance—the reticulin framework in the lymphoid and myeloid tissue, the reticular tissue in the liver (74). Due to the preservation of embryonic prospective potencies, they not only produce through proliferation monocytes, which can be looked upon as an easily transportable type of histiocytes, but they are closely related to lymphocytes or hemocytoblasts and, under certain conditions, may display full hematopoietic activity.

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THE ETIOLOGY OF DENTAL CARIES

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An historical résumé of the etiology of dental caries presents at least one interesting fact; namely, more has been written and less is known of this disease than any other dental lesion. An examination of the ancient Egyptian skulls shows that even the earliest races were not free from dental caries. It is true that some attempts were made from time to time to treat carious teeth by filling them rather than by extracting them, but the etiology, from the time of Hippocrates to the end of the eighteenth century, remained without significant development. The Hippocratic idea that dental caries was due to a certain stagnation of depraved juices was advocated by Bourdet, Bell and Serre as late as 1788. Other theories which held sway at different times were the humoral theory of Galen and the worm theory of Musitanus.

The modern concept of caries is due primarily to the work of Miller, who demonstrated bacteria in carious areas. The presence of certain factors is assumed to favor the development of the disease and their absence to account for freedom from it. However, as will be pointed out later in detail, the data thus far collected do not explain either the observed differences in caries or freedom from caries. In fact, the variations between the two conditions are, in most instances, too insignificant to justify any well-defined conclusions.

Since histo-pathology, chemistry, bacteriology, physiology and pathology all contribute their quota to a greater or lesser extent to the problem, it is thought best to consider the topic under the following separate headings:

1. Anatomical factors predisposing to caries.
2. The bacteria of the buccal cavity in their relation to caries.
3. The influence of the glands of internal secretion.
4. The relationship of saliva to caries.
5. Recent observations on the influence of diet on caries.

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Dental caries may be defined pathologically, as a progressive molecular disintegration of the elements of the tooth, beginning with the solution of the inorganic substances by the action of lactic acid formed within the mouth by fermentation, and terminating with the dissolution of the organic matrix through the solvent action or fermentative action of enzymes, or through products resulting from the activity of the saprophytic microörganisms.—MARSHALL, Operative Dentistry.

Although this is the generally accepted definition, it does not wholly present the facts of the case.

Caries is not to be confused with hypoplasia, abrasion, attrition or erosion. Hypoplasia is a condition of incomplete formation in which may be areas of soft tooth structure, or discolorations, or pits in hardened enamel. Abrasion, attrition and erosion differ markedly from caries in that these first mentioned diseases do not involve molecular disintegration of tooth structure, but rather are the result of a wearing away process caused by the wear of tooth against tooth (attrition), or of tooth against foreign body (abrasion); in the case of erosion, there is an obscure chemical process concerned in which the affected surface is always highly polished and without evidence of decalcification.

ANATOMICAL FACTORS. The normal tooth consists of four distinct tissues, namely: the enamel, dentin, cementum and pulp. The enamel is an extremely hard substance and consists mainly of complex calcium phosphates and carbonates, together with other inorganic salts in smaller proportions. The important chemical difference between enamel and dentin lies in the percentage of organic material; in the first instance, it is 3 per cent or less and in the second instance approximately 25 per cent. Histologically, enamel is composed of enamel rods bound together by an amorphous cement substance. The latter is the more soluble, for its loss in any area results in the loosening of enamel rods and subsequent cavity formation. Dentin is much softer, more resilient, and possesses, as its predominating histological characteristics, dentin tubules, fibrils and ground substance.

When caries develops on an exposed enamel surface, the acids supposed to be elaborated in the process first effect a slow solution of the cement substance, thus permitting the enamel rods to be washed away. At the junction between enamel and dentin, a much more rapid disintegration may occur and greater areas become involved than are apparent by an examination of the initial lesion at the surface. The dentin is next invaded by different types of bacteria which travel along the course of

the tubules, gradually decalcifying the amorphous ground substance between them. As the disease progresses, the organic matrix becomes exposed and putrefies until the entire crown of the tooth and the root portion are involved. Coincident with this the pulp becomes infected, unless recourse is had to operative procedures such as extraction or excavation and filling.

Burger has pointed out that carious teeth have a higher percentage of nitrogen than sound teeth and that this nitrogen is combined, not as nitrates or as ammoniacal salts, but rather as amino acids, uric acids or other organic compounds, probably products of bacterial metabolism.

Defects in the structure of teeth may frequently constitute an important predisposing factor in caries. Nutritional disorders of the mother during the latter months of gestation may be of such a nature that interference with the calcification of both deciduous and permanent teeth occurs. Instead of normal enamel or dentin, there may be areas of hypoplasia on the surfaces or in the substance of the tooth and these are liable to caries after the tooth erupts through the gingiva. Similarly, the exanthemata of childhood, since they are usually contracted during the periods of tooth formation and growth, may leave their mark upon the enamel surface as pits, honeycomb parts or deep fissures. These hypoplastic areas are more susceptible to caries than is normal tooth structure. Even the cement substance will show places of incomplete calcification and thus provide a favorable lodgment for microörganisms of decay.

Not only is the structure of the teeth concerned in this problem, but their position and relation to each other in the mouth also demands consideration. For example, the ill-advised early extraction of deciduous teeth delays or modifies the development of the dental arches. As a consequence, the position of the permanent teeth in both the mandible and maxilla is so changed that crowding or overlapping, particularly of incisors, cuspids and bicuspids results. This, as well, constitutes a factor toward the early development of decay, on account of the greater facility with which food particles collect between the teeth. Furthermore, the mechanical appliances used in correcting malocclusion or malposition of teeth, the clasps on artificial dentures, a filling incorrectly placed, or a poorly fitted crown, may contribute a considerable part in hastening the disintegration process. However, it is claimed by some authorities that these latter causes may be regarded as comparatively insignificant when scrupulous care in hygiene is observed.

On the other hand, it is an incontrovertible fact that malformed or malposed teeth do not always exhibit evidence of caries, but when they are attacked, the process may be more intensive than in those teeth which are without structural defect or which have erupted in proper alignment.

It is suggested by Cook that differences in the structure of teeth may be due to drinking water. In the case of those waters containing only minute traces of magnesium, calcium and other inorganic salts, there appeared to be a noticeable increase in the prevalence of caries in school children, whereas with those waters of either temporary or permanent hardness, the percentage of diseased teeth was somewhat lower. Forberg in Sweden and Rose in Baden (Germany) have reported similar results. It will be recalled that in Baden goiter is very prevalent, and the relationship between caries, on the one hand, and abnormal thyroids, iodine and drinking water on the other, is more actual than apparent and deserves more study.

Von Beust has claimed differences in the "vascularity" of dentin and enamel, and more recently Mummery has shown that there exist definite gradations in the degree of permeability of stains in young teeth as contrasted with older ones. He interprets this to mean that the calcification process may continue even after eruption of the tooth, a view which has been questioned by other histologists, but which nevertheless appears to be supported by the newer evidence presented by Mummery.

The structural defects which may be produced experimentally by imposing restricted diets upon animals will be referred to more particularly in that part of the review devoted to diet.

THE BACTERIA OF THE BUCCAL CAVITY IN THEIR RELATION TO DENTAL CARIES. The influence of microörganisms of the oral cavity upon dental caries has received attention by a number of investigators, for Miller, Magitot, Goadby, Black, Kligler, Howe and many others have all contributed important facts to this phase of the subject.

Although the bacteriology of the mouth is still undeveloped, there is one outstanding proven fact which may be mentioned—namely, that there has been demonstrated one group of organisms which is capable of producing and of resisting relatively high concentrations of acid upon tooth surface.

Miller was one of the first to announce that decalcification of enamel and the subsequent invasion of the decalcified mass by acid-forming bacteria may be produced experimentally when teeth are immersed

in cultures containing certain types of microörganisms. He pointed out in 1883 that decalcification of tooth structure precedes the liquefaction of the organic matrix and concludes:

> I do not, however, wish to be understood as saying, that acids or pathogenic bacteria, or putrefactive bacteria, or all together, are the sole and only cause of decay of the human teeth. What I am prepared to say is this, that in my opinion, there is not a single case of caries in which microörganisms do not play some part, and that in the most cases they play a very important part.

Later, he made chemical analyses of carious dentin and of dentin softened by acids and found that in both processes dentin "suffers a very great loss of lime salts", even the deeper layers of tooth structure showing this peculiarity. He sustained his theory that acids are produced in the mouth as a result of fermentation of food detritus and also that softened dentin owes its degree of decalcification to the local elaboration of acids by bacteria. His subsequent researches, reported in 1904, compared the relative rates of growth of one group of microorganisms, in culture media containing sodium bicarbonate, with another in which lactic acid was substituted. He observed that the greatest growth occurred in the tubes containing alkali after twenty-three incubation days, the growth being over 300 per cent greater than in the acid tube for the same length of time. The application of this to oral conditions is obvious, for saliva is comparatively rich in basic substances and when foodstuffs

> which are impregnated with alkaline saliva lodge between the teeth or in cavities of decay, a more rapid development of bacteria will take place in them than when the reaction of saliva is acid, the result being that the alkali is soon neutralized and a degree of acidity brought about equal to or slightly in excess of that produced by saliva that had an initial acid reaction.

Severe criticism of Miller's work was offered by Goadby and others, who drew attention to the fact that complete descriptions of bacteria were not given. In rebuttal, however, Miller mentioned that at the time this research was conducted solid culture media were only just being introduced and his methods of colony growth were confined to the older fractional system. Choquet took up the controversy, and after the appearance of various other polemical papers both Goadby and Choquet agreed that they had been unable to identify "a specific microbe of dental caries".

Meyer, in a critical review of the literature upon the microbiology of the mouth says that

it is of importance to emphasize that only repeated investigations will reveal the true significance of the oral microörganisms and that we certainly have not as yet completely succeeded in isolating all the microörganisms seen microscopically in the buccal cavity.

Kligler classified the organisms of dental caries in accordance with the successive stages of development of the disease. In the primary and secondary lesions in which enamel rods are separated from cement substance and dentin invaded, the *B. acidophilus* (Moro), the *Cladothrix placoides* and the *Leptothrix buccalis* constitute from 40 to 60 per cent of the oral flora. Although the *Cladothrix* produces less acid than the *B. acidophilus*, it possesses the property of fixation of the acid producing flora to the tooth surface. "Kligler found in plate colonies, that a network of *Cladothrix* threads sometimes enclosed one or two colonies of other bacteria" (Meyer). Similarly, the *Leptothrix* is capable of adhering to smooth tooth surface and its growth is favored by the salivary mucin and contained salts. In the third and fourth stages of the disease, in which the solution of the inorganic salts exposes the dentin matrix and thus eventually reaches the dental pulp, the *B. acidophilus* is present to the extent of 30 per cent, the *B. putrificus* from 10 to 20 per cent and cocci from 40 to 50 per cent.

As the lesion penetrates deeper and deeper toward the pulp, the *Cladothrix* gradually disappears so that in the later stages, when the pulp has been exposed and has become putrescent, the character of the flora alters to a considerable extent. The thread forms are no longer demonstrable and another organism, "an anaerobic putrefactive spore bearing rod, the *B. putrificus*, is found in large quantities. This organism corresponds with the one described by Rodella, who attributed, however, the entire cycle of decay to this organism. Possessing powerful proteolytic properties, this bacterium is—according to Kligler—important in the process of pulp decay" (Meyer).

Lothrop and Gies investigated "the influence of acid-producing bacteria in the focal precipitation of salivary mucin under relatively large masses of unaffected colloidal matter." Their conclusions were based upon recorded observations made with litmus-glucose-gelatin and they found that the acid was capable of precipitating mucin in the immediate vicinity without rapidly diffusing away from the place of fermentation or of disorganizing the overlying protective mass.

Gies, in commenting upon the work of Kligler, points out that these researches do not explain immunity to caries, localization of caries, or the sudden halt in the lesion. This latter condition is accompanied

by the reparative process, in which there is an apparent "recalcification" of areas previously decalcified. It has also been found that the *Cladothrix* and the *B. acidophilus*, isolated from a case of immunity, exhibit just as marked decalcifying properties as do those organisms from cases of true caries.

Howe, working upon the Moro-Tissier type of microörganisms, confirmed the fact that this group is the predominating one in dental caries and further that it is capable of producing such relatively high concentrations of acid that it limits automatically the elaboration of other types of bacterial flora.

Eckermann presents a somewhat novel theory of the etiology of dental caries and ascribes a rather minor rôle to microörganisms. His concept of the disease differs from that held by American and English investigators, for he believes that dental caries possibly occurs in two stages; the primary, which may be termed perhaps the physiologico-pathological stage, and a second stage in which tooth substance is attacked by bacteria. In the first instance, he considers the effects which may be produced by osmosis between saliva and blood serum, assuming that the osmotic "membrane" consists of dentin and enamel. When concentration of crystalloids in saliva is higher than that of blood serum—a condition which may occur—osmosis is said to be induced between the blood serum contained in the blood capillaries of the pulp on the inside of the tooth, and the oral fluids on the other side.

The histological data presented describe a cone-shaped area of colored dentin with its base toward the carious enamel and its apex toward the pulp. In conditions of malnutrition, where there has been faulty formation, and where hypoplasia exists to any marked degree, it is conceivable that osmosis might occur with greater ease than in those conditions in which tooth formation has been entirely normal. The osmotic action between the two liquids is not, according to Eckermann, in the nature of a decalcification, but rather provides a nutrient medium favorable for bacterial growth. It is here that part two of dental caries begins, for it includes a consideration of the decalcification of enamel and dentin as brought about by the organisms usually concerned with this process.

It might be pointed out, in discussing Eckermann's theory, that there are certain clinical facts in relation to dental caries to which he has not accorded their respective values. For example, he notes that at death, dental caries stops and from this assumes that microörganisms

may play only a minor rôle in the elaboration of the disease. In a discussion with one of his reviewers he writes: "The hypothetical knowledge of the origin of dental caries being caused by lodging fermented carbodydrates has in no direction helped us to stop the increasing wave of decay". Eckermann says, in defense of his observations upon the lack of progressive decay of teeth of skeletons, that

> it is a singular fact that whereas the body, buried in the earth, will quickly be completely annihilated, the teeth, on the contrary, offer for many thousand years a resistance against decomposition. Among these teeth some may, also, be carious. Death has stopped, instead of hastening, decomposition of the teeth, whereas, in conformity with the hypothetical chemico-parasitic theory, we should have expected the contrary, independently of the question if parasites or saprophytes are the cause of the annihilation. The destruction of the teeth seems, consequently, to be dependent upon a natural power which is irrespective of the common chemical nivelation process of animal life.

This appears to the writer to be a rather secondary consideration for, after all, we are concerned primarily in eradicating decay of teeth in children rather than in skeletons.

Additional information upon this subject has been given by MacIntosh, James, and Lazarus-Barlow. After an exhaustive examination of selected carious material, the authors were able to differentiate a definite type of bacillus which is capable of producing a high degree of acidity by the fermentation of carbohydrates. "The average pH value of nine strains was 2.75, which is sufficient to decalcify teeth." It was also shown that teeth left in contact with pure cultures over prolonged periods show changes almost identical with those found in natural caries.

Although the organism is similar in some respects to the *acidophilus* group of *Moro*, there are certain biological differences which they deem of sufficient importance to warrant a separate classification. The term "*B. acidophilus odontolyticus*" is proposed and this in turn is divided into two types, the first of which resembles closely organisms illustrated by Miller and apparently identical with those described by Howe and Hatch.

The sugar reactions reported by MacIntosh, James, and Lazarus-Barlow were found to be similar to those of Kligler in 60 per cent of the cases. And finally, these authors confirmed Miller's observations, made over forty years ago when the science of bacteriology was in its infancy, that the first sign of caries is loss of transparency of enamel.

The importance of the *acidophilus* group of organisms to the general health has been stressed by Rettger and Cheplin. They have shown

that the effects in the intestinal tract, supposed to have been due to *B. bulgaricus*, are in fact caused by *B. acidophilus*. If this latter organism is so necessary for some individuals, one hesitates to infer what will be the result on the teeth when *B. acidophilus* is administered internally, particularly when it is recalled that Dieterlen demonstrated the presence of organisms in the mouths of experimental animals one hour after their injection into the lower bowel.

Howe and Gillette agree with the findings of Kligler relative to the type of bacteria found in caries. Although the uncared for mouth, regardless of the presence or absence of caries, contains a greater number of organisms than the clean mouth, the *type* of organism is apparently the more important factor.

From our present knowledge it may be deduced that the *streptococci* are found to a lesser extent in all stages of decay than in immunity therefrom, but that the acid-producing organisms exhibit a marked increase. This may be explained by the fact that under ordinary conditions of cleanliness, the mouth is freer from proteins than from carbohydrates and that therefore the medium is unsuitable for the development of proteolytic flora. However, in areas of decay this condition is changed and the exposed protein of the organic matrix in the tooth provides a more favorable condition for the production of the symbiotic relations between the anaerobic and the aerobic organisms. As a result, the degree of acidity varies and in fact increases with the development of putrefactive processes resulting from the breaking down of proteins.

In concluding this section, it is apparent that bacteria are not the only factors concerned in dental caries. It is known that the *B. acidophilus* is demonstrable in every mouth without regard to the presence or absence of caries. Further than that, the acidophilic properties of different strains obtained from carious and non-carious conditions are the same. It appears, therefore, that both the environment and the tooth structure are more important factors than the types or numbers of organisms concerned. The kind of food, fibrous, or non-fibrous, the efficient cleansing of tooth surfaces, the general health of the individual, the degree of nutrition,—all of these factors are concerned in an etiology, formerly considered almost exclusively from the bacteriological standpoint.

THE INFLUENCE OF THE GLANDS OF INTERNAL SECRETION. It has been suggested from time to time that malfunction of the "ductless glands" exerts an influence upon the formation of teeth and that there-

fore it may also be a factor in dental caries. This present viewpoint has been developed, in a measure, from the study of an experiment in quite a different field.

In 1906 Erdheim destroyed portions of the parathyroid glands in rats by thermal cautery, and those animals which survived the operation long enough developed horizontal grooves on the labial surfaces of the upper incisor teeth. It was subsequently shown that these grooves were the result of an interference in calcium metabolism occurring at the time when these particular teeth were being formed in the jaw. Although Erdheim's interest centered about the phenomena of calcium metabolism, his experiment suggested the possibility that those conditions which produce changes in the deposition of calcium salts in bone may likewise be responsible for analogous effects in the growth of teeth. The clinic has contributed additional evidence of this relationship, for Howard, in discussing hyperthyroidism says: "The teeth show rapid decay and marked fragility."

Little experimental work germane to the subject has been reported since 1906, although both Waller and Broderick deduce certain theories relative to the influence of the ductless glands upon the etiology of dental caries. Halliburton, in discussing Broderick's paper, has protested against some of the generalitites which have been made and believes that it is premature to explain how calcium metabolism may be interfered with or may be influenced by either endocrines, vitamines or acidosis. In fact, Halliburton intimates that Broderick's authorities included only those whose views fitted in with his (Broderick's) theories. The justification for the pluriglandular therapy of Broderick has at present but scant support from the experimental evidence at hand.

The researches having the most direct bearing on the subject are those of Gies and his co-workers, in which two sets of experiments were conducted, a surgical one and a dietary one. They have reported that in rats there is no effect on dentition following thymectomy, thyro-parathyroidectomy and castration, but that deficient calcification of the incisors was observed after parathyroidectomy. In the second experiment, white rats were fed with various tissue preparations and the effects observed of such a diet upon the calcification of the teeth. It is stated that there was a decreased calcification when lymphatic, salivary or thyroid gland was administered, an increase with testicular tissue, and no effect from corpus luteum, parathyroid, pineal, pituitary, spleen, suprarenal and thymus.

Although Pickerill has suggested that there may be osteo-genetic or dento-genetic hormones present in the body which are in some way related to calcium metabolism and to the utilization of calcium by the body, he offers little proof in substantiation. The literature, in fact, is replete with various views, but unfortunately the recorded observations dealing with dental defects are so scant and difficult of access that comprehensive data have not been compiled nor has the dental aspect received the attention which it merits.

THE RELATIONSHIP OF SALIVA TO DENTAL CARIES. Researches dealing with the changes produced by saliva upon the development or the retardation of dental caries were reported by Magitot in 1878. He deduced that

> the saliva is the veritable agent of caries not indeed by its original constitution unfitted of itself to exercise any action on the teeth, but in consequence of the infinite variations its composition undergoes under adverse influences, which we shall examine; variations resulting either from the spontaneous development of substances endued with an altering action upon the dental tissues, or from the accidental introduction of these substances, already formed, from without.

The degree of alkalinity and acidity of saliva he thought was variable and that this variation was due entirely to abnormalities in glandular activity. For

> the various elements composing the mixed normal saliva have then a primitive alkaline reaction, and the acid conditions must come exclusively from the fact of certain changes or fermentations, not indeed of these elements themselves, but of foreign substances introduced accidentally, or by the alimentation, and which in this manner give rise to acid products.

He differentiated also between those conditions in which saliva was viscous and the mucin abundant and those in which the opposite obtained.

> If the mucus is abundant and the saliva viscous, the surroundings of the teeth will be acid and the tartar scarce or entirely wanting, this substance being dissolved as soon as formed; but there should be found in such mouths more or less numerous caries of a whitish color, soft and of rapid progress. If, on the other hand, the mucous deposit is entirely wanting, we shall find there a local alkaline reaction, the presence of tartar in great abundance with its consequences upon the gingival border, and a relatively smaller number of caries, or their complete absence.

The above modifications of the oral fluids were observed to a greater degree in cases of illness.

By morbid conditions of the saliva we understand the changes which the influence of pathologic conditions brings into its secretion or into its composition, and are classified under:

(a) local affections of the mouth and of the pharynx;
(b) general acute diseases;
(c) general chronic diseases.

The author emphasizes the fact, often observed, that hyperactivity of the salivary glands is a symptom of various forms of stomatitis and believes that changes in the chemical composition of the fluid are such as to favor the development of caries. Similarly, in the general acute and chronic disorders such as are evidenced, for example, by febrile conditions, there are variations in both the amount of secretion produced and in the proportion of the substances contained. For example, in enteritis, dysentery and dyspepsia

their effect is to exaggerate the proportion of ptyalin, the agent of viscidity and of fermentation, and to excite a hyper-secretion of mucus, two phenomena caused either by the direct influence of intestinal alteration, or by the reflex action of the general condition upon the conditions of the mouth.

Some of the later investigators, as, for example, Quincerot in 1909, Kirk in 1919, Lohmann, Bunting, Howe, Gies and collaborators, Jeanneret, Hopewell-Smith, Zwimpfer, Felder, Prinz, Berger, Caluori and others, have discussed saliva from somewhat similar grounds. Quincerot stated than an unclean mouth constitutes a predisposing factor in the production of dental caries and that dry mouths are immune to caries. Mouths in which there is abundant saliva, especially if the latter is viscous, "will surely exhibit signs of caries." Although this report was published as late as 1909 there is little laboratory evidence presented to support all of the author's statements, especially insofar as saliva is concerned.

Kirk, in a rather comprehensive review of experimetal work by other investigators says:

The conditions which determine the factor of susceptibility or of immunity to dental caries are those which determine the composition of the mixed oral fluids which we call the saliva. In the absence of a bactericidal element in the saliva, which fact may be regarded as proved, I think we are justified in our present state of knowledge in viewing immunity to carious action as being dependent upon the absence from the saliva of the kind and amount of pabulum upon which the bacteria of decay normally thrive.

The author further states that the composition of saliva may possibly depend upon food habits and that the desired immunity may be attained only through a more intelligent and rational dietetic hygiene.

The problem of the sulfocyanate content in saliva is one which has been of great interest and caused an unusual amount of discussion in dental literature. Lohmann assumed that the "sulfocyanate of sodium" suffers a decomposition by means of the hydrochloric acid in the stomach and that this decomposition results in the formation of sulfocyanic acid and sodium chlorid. Although this cyanogen compound is bactericidal, its effect is fugitive, for its breaks down at once into more stable substances. He recommended that a solution of sodium sulfocyanid, 2.6 grams in 10 cc. of water, be administered to patients exhibiting rampant caries. He claimed that the drug prevents decay of deciduous teeth and allays pain.

Bunting reports upon this same topic and draws a comparison between the content of potassium sulfocyanate in saliva and immunity to dental caries. When a salivary analysis indicates a deficiency of this compound, and dental caries is present, it is recommended that the deficiency be met by medication with sulphocyanate. When Gies reported in 1913 upon the problem he doubted that the compound possesses any inhibitory action upon dental caries and also emphasized the unscientific and dangerous practice of its indiscriminate administration by dentists. As a result of Gies' researches the practice of treating dental caries in this manner quickly fell into disrepute.

Jeanneret performed a number of determinations on uric acid in saliva. His researches indicated that the relationship of nitrogen to uric acid is not a constant and that it varies between 9.8 and 54.1. These variations were found not only in healthy individuals but also in those patients who suffered from tuberculosis and from different diseases of the nervous system and the circulatory system.

Further research upon this subject is reported by Felder. He found that saliva contains approximately 0.1638 per cent nitrogen and 0.343 per cent albumen. The nitrogen and albumen content do not parallel each other, and in addition it was shown that the percentage of the salivary nitrogen obtained during fasting did not differ from that secured from a well-nourished individual. On the other hand, the content of albumen in fasting saliva, compared with that obtained with a "full diet," appears to be somewhat lower. The albumen nitrogen averages only 33 per cent of the total nitrogen in saliva, the greater part being found in other nitrogenous bodies. He believes that the administration of calcium chloride produces a saliva low in albumen, and the same results were found to be true in the case of the milk diet. On the other hand, sodium chloride and sodium iodide tend to raise the albumen

content a trifle and the latter further increases the nitrogen content. With the carbodydrate diet, the nitrogen content falls below the average, whereas the albumen content maintains a normal level.

Hench and Aldrich more recently have determined the combined nitrogen and urea content in saliva and have correlated their results with abnormal renal function. The high nitrogen values of saliva indicate the necessity of studying in greater detail the nitrogen values of blood. The saliva-urea index is a constant under normal conditions, but in the event of urea retention, the index increases in definite parallelism.

Prinz has discussed the problem from the standpoint of enzyme content of saliva. He has pointed out that the pH value of saliva influences the activity of the various enzymes found. The hydrogen ion concentration figures agree very closely with those obtained by other experimenters. Howe believes that "saliva having a high phosphate determination, alkaline, and with low chlorides, is ideal for carbohydrate fermentation on account of the dibasic sodium phosphate present."

Brown, in discussing the degree of acidity and alkalinity of culture media, has very clearly demonstrated that a knowledge of the buffer content of media is as important as that of the hydrogen ion concentration. The buffer index is the sum of reserve acidity and reserve alkalinity. Since the oral fluids constitute an important culture media for the propagation of the oral flora, the knowledge of the buffer index, as shown by Marshall, is as important in this case as it is in the laboratory study of bacterial growth.

Pickerill's work on dental caries, first published in 1912, deals with the problem from the standpoints of histology, chemistry of saliva and dietetics. He emphasizes, among other things, the importance of psychological interference and influence upon the salivary activity by quoting at length from Pavlov, Schaefer and Starling. Mechanical irritation to the oral mucosa usually gives rise to an increased salivary flow, an abnormality which may hardly be overlooked in determining the constituents of normal saliva. Pickerill correctly observes that the quantity of saliva produced by an individual in a given unit of time is a necessary factor for determining relative chemical differences in various salivary samples, but the method which he employed and described does not bear careful scrutiny. For parotid saliva a cannula of german silver tubing was used and for sub-maxillary and sub-lingual saliva, a vulcanite appliance was so constructed that the outlines

followed the floor of the mouth and the inner portion carried a small tube through which the secretion flowed. It is to be noted in this connection that the placing of a cannula in a duct, or the insertion of an appliance around the lingual surfaces of the lower teeth provides in itself a mechanical stimulus in the form of an irritation which is difficult to evaluate and which is undesirable to initiate, for saliva so collected cannot be said to have been produced under normal conditions. However, the method provides at least a separation of the parotid fluid from the sub-maxillary and sublingual fluids which may or may not be of subsequent interest. A number of determinations of salivary alkalinity were made, under various conditions of "normality" and "abnormality." It is regrettable that the method of making these determinations is open to the serious criticism that it lacks accuracy. Pickerill used fiftieth normal sulphuric acid with methyl orange as an indicator, but Sutton, Tredwell-Hall, Schmif and many others agree that this indicator is unsatisfactory in solutions weaker than tenth normal, on account of the fact that the change of color accompanying the conversion from acid to base, or vice versa, is so slight and so gradual that the eye can detect it only with the greatest difficulty. On this basis, the alkalinity index, which is stressed throughout the book, is not an index at all on account of the fact that in weak solutions "an excess of 0.3 cc. of tenth normal alkali is necessary to change from red to yellow" (Tredwell-Hall). With fiftieth normal solutions this inaccuracy is of course multiplied by five and becomes 1.50 cc. His experimental error is, therefore, of greater magnitude than his titration figure, for in normal saliva the titration figure is only 1.05 cc., which is 0.45 cc. less than his experimental error. Therefore, the "alkalinity index" even for the normal individual may vary anywhere from 1.73 to 2.49.

Another research bearing upon the problem is that reported by Marshall in 1914. Prior to this, the estimation of the salivary reaction depended entirely upon the degree of acidity or of alkalinity and it had been shown by Gies and many other investigators that these determinations, considered separately, varied within too wide a range to prove any relation to oral conditions. In fact, the magnitude of the variation, even in one individual, is too great to indicate significant deduction. Marshall reported, however, that under a certain set of standard conditions the saliva possesses a neutralizing power which is similar to the buffer action of the blood. This was estimated by measuring the quantity of reagent required to change saliva from one definite

reaction of faint alkalinity to another definite reaction of faint acidity. Upon stimulation of the salivary glands, this neutralizing power may remain nearly constant or it may increase to a remarkable degree, depending in a measure upon the presence or absence of dental caries in the individual from whom the sample was obtained.

The origin of this change was found by dialysis experiments to be the result of a difference in the concentration (in the case of stimulated saliva) of the inorganic salts,—the greater the concentration the greater the neutralizing power. Furthermore, these diffusible substances bear an inverse ratio to the non-diffusible ones; for example, the amino nitrogen determinations of the two salivas in caries (non-stimulated and stimulated) show less variation between them than do those in the condition of freedom from caries.

There is evidence, also, that the relationship is independent of lesions of the central nervous system, for in cases of dementia praecox and epilepsy the changes in salivary reaction are similar to those observed in normal individuals. Local stimulation of different areas in the mouth resulting from electric stimuli, or from taste stimuli, do not produce as great a change in neutralizing power as does the mechanical stimulus provided by chewing paraffin.

Other experimenters, in attempting to repeat Marshall's work, have encountered certain difficulties in securing average samples, and in carrying out the subsequent determinations, for a strict observance of all the details is an essential part of the work and any lapse or oversight in technique invalidates the experiment. Howe, for example, showed lack of attention to technical detail, as did Bunting. It is not sufficient to use merely freshly distilled water to dilute saliva—the water must not only be freshly distilled but it must, in addition, have been recently boiled and cooled to rid it of the CO_2 in solution. It must be, in fact, "conductivity water." Further than that, the samples were taken at almost any time of day without due regard to the points mentioned by Chittenden and Richards that there is less variation in salivary secretion between the hours of seven and eleven in the morning. In addition, it may be stated that the use of control tests to check the ability of the operator in getting the correct end point is not mentioned, nor does one find anywhere any reference to the use of a color guide as an aid in determining the end point. Gies and his coworkers have also attempted to repeat some of this work and chose to deduce certain statements by comparing salivas secured by various *different* stimuli instead of by the *same* stimulus.

The hydrogen ion concentration of saliva has been studied by Prinz and others but it is to be pointed out that *buffer value* is the index sought rather than actual acidity. For this determination the older titration method seems better adapted than the one involving the hydrogen electrode, as shown later by Brown in the study of culture media. It has been suggested by Marshall that the differences in buffer value between unstimulated and stimulated saliva in immune cases may exert an inhibitory action upon the development of bacterial flora in the mouth, whereas in caries both the salivas are nearly the same and therefore favor bacterial activity. This suggestion seems to have a particular application when one reviews the work of Kligler on the oral microörganisms.

Recent observations on the influence of diet on caries. The earlier observations upon diet, aside from those of the ancient Egyptians and Greeks, dealt with the effects of fibrous and non-fibrous foods upon the teeth, a subject latterly treated at some length by Edsill, Sims Wallace, Pickerill and others.

Both the physical and chemical properties of food have been shown to influence the function and health of teeth and presumably, therefore, these properties are important when the predisposing factors of dental caries are considered. Soft diets, although necessary in illness and for very young children, tend to relieve the masticatory apparatus of the usual amount of exercise in chewing. Under these conditions, the teeth and the supporting structures suffer from a lack of sufficient use, the result of which is to be found in underdevelopment of the jaws with a subsequent crowding of teeth. This malposition invites carelessness of oral hygiene on account of the difficulties in cleansing all of the tooth surfaces. Orthodontists, in correcting malocclusion, stress the importance of function, for growth of jaws is favored by well directed exercise. The physical characteristics of food, therefore, bear a relation to caries, for soft diet promotes disease, whereas the fibrous one, in addition to its stimulating effect upon the process of mastication, serves also, in a measure, as a cleansing agent on the surfaces of the teeth by virtue of the fact that during the process of comminution the particles are moved backward and forward over all the exposed dental surfaces.

One should not overlook the fact that the relatively recent processes of *refining* such foods as, for example, wheat flour and white sugar, together with their increased consumption, has brought about the adoption of an unnatural diet insofar as purity is concerned. The bran and wheat germ, the brown molasses, so scrupulously removed in processing,

have all been found to contain substances of nutritive value, yet they have been discarded as articles of daily diet.

Frick early called attention to the fact that the artificial feeding of infants may be a predisposing factor in decay among the newborn and later stated that the main cause of dental degeneration is faulty nutrition. In fact, he goes so far as to conclude that the predisposition to dental caries increases at the same ratio as bottle feeding. His statement concerning the Chinese and Japanese, that there is less incidence of metabolic disease and of dental caries among them, needs revision, in view of our more recent knowledge on the subject.

Although there have been many reports upon the anthropological aspect of the incidence of dental caries, the authorities are not in entire agreement. Mummery has pointed out that an examination of carious teeth in the skulls of some of the primitive races discloses a variation of from 1 to 0.4 per cent in the Eskimo to 20.8 per cent in the Negro. This ratio, among the civilized races, has increased in many instances to over 90 per cent.

Kirk reviews some of the statements of Miller bearing upon the degree of fermentation occurring in a given time in saliva. When the sample is drawn from an individual who is susceptible to dental caries, fermentation occurs sometimes to such an extent that a relatively high acidity is obtained. He also quotes Cushing to the effect that

> Doubtless comparable illustrations in sugar tolerance occur in other periods in physiological readjustment. It is not unlikely that the glycosurias of adolescence may prove to be coupled in some way with deviations in the internal secretions which are on the border line of the physiological normal. In view of the occurrence at this period of life of a rapid increment, particularly in skeletal growth, it is a natural conjecture that these glycosurias may be related as closely to hypophyseal hypoplasia as to the more obvious changes of the interstitial cells of testis and ovary which occur at this time. Both the facts already established make a sufficiently connected story to justify the avoidance of speculation.

It is apparent that there is more uncertainty at this time concerning the relation between caries and the chemical composition of food than formerly. The added impetus to the study of nutrition which has been given by the discovery of the vitamines and their attendant effects has thrown new light upon the dental aspect. The views of Miller upon the disease have not been overthrown, but his concept of caries is gradually being modified by the fact that experiments have been reported in which "caries-like lesions" have been produced experimentally.

Howe describes a research on rats and monkeys in which abnormal teeth were produced by imposing restricted diets, the restriction consisting mainly in lack of anti-scorbutic vitamine. He says:

> Feeding diets that are deficient in respect to their vitamines does produce decay of the teeth in young animals and in some of the older animals. Diets deficient in this respect also produce carious areas in the process and in the jaws. In some cases the entire bones of the head were carious. It makes no difference whether these disturbances are the result of an irregular calcium metabolism or the result of an infection, the control lies in the diet.

Further than that, he has claimed that recalcification occurred when the green food of the diet was increased. The author also takes issue with Miller and points out that some of the conclusions of Miller relative to the etiology of dental caries do not appear to be entirely justified. This is based upon the fact, first, that the magnifications which Miller used to show microörganisms are insufficient, and he also points out that Miller "was never able to produce caries, according to this theory in animals."

In conducting studies of the vitamine A avitiminoses on puppies, Mrs. Mellanby describes unusual conditions of bone formation, tooth formation and tooth position. She says: "Fat soluble A (or something with a similar distribution to that substance) is one of the principal factors involved in the normal growth of teeth and jaws." And in her second paper, that

> (1) there is a direct relationship between the structure of the teeth and caries, those badly formed having greater susceptibility and those well formed being more immune to this disease.
>
> (2) The structure of human teeth is probably controlled by dietetic factors in the same way as shown experimentally in the case of dogs.

Although these statements might be considered as somewhat dogmatic, it may be accepted as proven that, in experimental animals at least, deficient diets, if severe enough, eventually affect the formation and structure of the teeth as well as the bones.

An exhaustive study of the effects of different dietary elements upon the teeth has been made by McCollum and co-workers. Although the clinical findings are interesting, and without precedent up to the present time, the application of the material to the consideration of the etiology of dental caries is not yet apparent. However, the oral defects are described as "caries-like lesions, pulp exposure, osteo dentin, fracture and overgrowth of teeth."

The type of diet which produced the greatest percentage of dental abnormalities was one in which there was a deficiency of "protein, calcium, and fat soluble A." The next highest incidence of defective dentition was exhibited in rats having low calcium, and the third percentage in those rats low in calcium and fat soluble A. The oral tissues were least damaged by three types of diets; namely, those which were high in calcium and low in fat soluble A; second, those which were high both in calcium and col liver oil vitamine; and third, those which were low in calcium and in cod liver oil vitamine.

Although the authors decline to discuss the possible relationship between anti-neuritic and anti-scorbutic substances to oral disease, yet it is possible that a deficiency of the anti-scorbutic in man would constitute a factor in the etiology of oral disease. But it is pointed out that the rat is unlike man in being able to synthesize within its own economy that particular protecting substance. Severe dental disturbance may follow a diet which is only relatively defective. In fact, it is apparent that the manifestation of the disease is out of all proportion to the extent of the deficiency.

The authors conclude that

> it is not possible at this time to name any one deficiency which specifically causes dental or oral disease; it would appear that any slight variation in the American diet, which always so dangerously approaches the level of dietary deficiency, might become active at any period of lowered resistance or of physical or nervous stress.

Dental abnormalities of a nutritional origin have also been reported by Marshall. He found that dogs maintained on deficient diets for different periods of time give evidence of profound alteration of tooth structure, for instead of normal dentin, a highly vascular, fibrous tissue—a so-called "dento-pulp"—has been demonstrated. Although there have been no cases observed up to the present time in which teeth of these experimental animals showed signs of caries, yet it is probable that the dietary deficiencies, as reflected in abnormal tooth structures, are correlated to dental caries.

Future research upon the etiology will probably develop rapidly, at least in two main channels. One may look with interest toward the elaboration of experimental data upon the anatomical changes occurring in teeth as a result of deficient diets, and second, upon a possible relationship between certain salivary constitutents which either predispose or inhibit the development of dental decay. The insidiousness of the onset of the disease, the difficulties which are frequently experienced

in controlling it, and the unknown factors which influence its cessation, all are still matters of conjecture, and until the complete etiology has been worked out the treatment will be one of repair rather than one of prevention. It appears, therefore, that caries is at least a twofold process in which defective structure of enamel and dentin is closely correlated with deficiencies in diet and physiological abnormalities. Further than that, certain types of bacteria found in the mouth elaborate locally a sufficient amount of acid to decalcify tooth structure and in this manner form cavities. The factors tending toward an arrest of the process, however, have not as yet been recognized.

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A REVIEW OF ACUTE EXPERIMENTAL NEPHRITIS

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There are not many fields of medical thought in which the experimental method of study has been more extensively employed than in investigating the various problems of acute nephritis. This has resulted in the accumulation of an ever increasing amount of reference material.

In reviewing this material it has been interesting to note the development of research methods. The earlier studies were, with few exceptions, purely anatomical in character. At a later period such studies concerned themselves with the ability of the kidney in which different types of injuries had been induced to form urine. The chemical composition of the urine was studied by comparatively superficial methods. Later on investigations were undertaken of a broader and more complex nature which included not only the response of the nephritic kidney to various diuretic substances, but of its ability to eliminate various products of metabolism, and finally, by a study of those changes in the blood and tissue juices which such injuries effected.

An attempt to follow up in this review the many important avenues of research which have been developed through experimental studies of acute nephritis would lead the reviewer very far afield. For this reason the following review only concerns itself with the anatomical and functional changes induced in the kidney by experimental methods and with those changes in the organism as a whole which should be considered as an essential part of such renal injuries.

For purposes of convenience, and in order that the review may be of more use to others, the various investigations have been classified under the heading of the nephrotoxic substance which was employed to induce the nephritis.

The work of others who have been interested in such compilations has been freely drawn upon. This is particularly true for the papers and monographs by Pearce (1), (2), (3), Sollmann (4), Christian (5), (6), (7), (8), (9), Christian, Frothingham, O'Hare and Woods (10), Ophüls (11), Ewing (12), Sellards (13), Cushing (14), Karsner (15), Aschoff (16), Fischer (17), (18) and Loeb (19). In addition to these liberties I have,

in the use of several of the more important papers, largely quoted the conclusions of the respective authors, with the feeling that their interpretation of what they had accomplished would be of more value than an incidental abstract.

I. ACUTE URANIUM NEPHRITIS. Likely on account of the early observation of Leconte (20), which was later confirmed by Richter (21) (22) and others, that uranium would not only induce a renal injury but that it would lead to the development of a general anasarca, this substance has been more generally employed to induce both acute and chronic types of renal injuries than any other chemical substance.

Chittenden and Hutchinson (23) and Chittenden and Lambert (24), (25) not only investigated the inhibitory effect of uranium salts on ferments, but they observed that in the early stages of their action as kidney poisons the amount of urine was increased and that the animals became glycosuric. The late action was characterized by the development of a parenchymatous type of nephritis with a partial or complete arrest of urine formation. The renal injury according to these investigators closely resembles that induced by phosphorous. Both the epithelium and vessels were involved in the injury. Ascites was frequently observed.

In the studies of the acute nephritis induced by uranium many of the investigations have been concerned with both the localization and type of its action in the kidney. Christian (26), Christian, Smith and Walker (27) and Christian and O'Hare (28), working largely with rabbits, described the epithelial injury induced by uranium and noted the occurrence of hyaline droplets in the walls of the glomerular capillaries which were later followed by more advanced degenerative changes in the glomeruli. Such changes could not be demonstrated in other small vessels outside of the kidney, such for instance as those of the heart and spleen. The late glomerular changes consisted in the formation of hyaline thrombi, hemorrhage, hemorrhage into the glomeruli with dilatation of the subcapsular spaces and finally the proliferation of capillary endothelium and capsular epithelium.

Schirokauer (29) and Heineke and Myerstein (30) in their work with rabbits induced both severe vascular and epithelial injuries with uranium and noted the occurrence of the edema. Pearce (31) classes uranium with the chromates and considers the early damage to the kidney as largely tubular and confined to the convoluted tubules. In a later paper O'Hare (32), working with dogs as did Pearce, pointed out the difficulty of inducing glomerular changes in the dog kidney. From the anatomical

studies of the action of various nephrotoxic agents on the renal epithelium MacNider (33) concludes that in the dog this tissue is the early seat of the uranium action and that only after several days of such an intoxication do the glomeruli participate histologically in the injury. It would appear that the glomeruli of the rabbit are much more susceptible to injury from uranium than is the case with the dog.

Aschoff and Suzuki (34) have greatly advanced the study of the selective action of different nephrotoxic substances by employing a method of vital staining and then subjecting the kidney to the action of different poisons. They were able to show that different portions of the convoluted tubule exerted a selective affinity for different poisons. By such a method they divided this portion of the renal tubule into three portions and demonstrated in an acute uranium nephritis its selective action for the third and distal portion.

Their studies enabled them to study the repair of the kidney following this poison. A regeneration of renal epithelium occurred from the upper portion of the descending limb of the loop of Henle and in this manner replaced the degenerated cells in the terminal portion of the convoluted tubule. Oliver (35) confirmed many of the observations of Aschoff and Suzuki and studied in detail the histogenesis of chronic uranium nephritis. In a later paper (36) which was concerned with both histological and functional observations, Oliver employed rats which were fed on a mixture of lard and corn meal containing urea. In other animals a solution of urea was injected into the peritoneal cavity. When the animals were secreting a urine high in urea they were killed and injected with xanthydrol which forms with urea a characteristic crystalline product. A microscopic study of sections from such kidneys showed urea present in the cells of the convoluted tubules in far greater concentration than in the glomeruli or in any other portion of the tubule. The experiments strengthen the conception of the secretory value of the tubule.

In a later paper than the previousiy referred to anatomical study, Aschoff (37) studied the changes in urine secretion under normal and pathological conditions. Uranium, cantharidin and bichloride of mercury were used as nephrotoxic agents. He obtained from these substances swelling, hyaline vacuolation and necrosis of the tubular epithelium, and contrary to most observers was able to induce tubular lesions with cantharidin without severe involvement of the glomeruli. He considered the division of renal poisons into tubular and vascular not justifiable. Investigations by Suzuki (38) and also by MacNider (39) emphasize

the ease with which the proximal convoluted tubule may be injured by a variety of poisons. In the latter study potassium dichromate, uranium nitrate and sodium arsenate were found to produce an acute nephritis in which both the vascular and epithelial elements of the kidney were involved. Uranium and the chromates produced a tubular injury much earlier than cantharidin or arsenic. In the early stages of the injury from all of the poisons when there was only marked congestion of the glomeruli and no evidence of tubular injury the formation of urine was decidedly increased. At a later stage when no change had taken place in the glomerular pathology, but when the epithelium had become injured, the output of urine was reduced or an anuria was established. Arsenic was found to induce the most marked injury to the glomeruli which was in general out of proportion to the tubular damage. Schlayer, Hedinger and Takayasu (40) in their anatomical and functional studies of the nephritic kidney support the above findings concerning the dominant action of uranium on the tubules but they also show that in a uranium injury the glomeruli may be functionally inactive even though there is little evidence of histological injury.

An attempt has also been made by Boycott and Ryffel (41) to classify various nephrotoxic agents by a combination of both anatomical and functional studies. In this study they group as primarily vascular, cantharidin, arsenic and diphtheria toxin, and as primarily epithelial poisons, especially inducing injury to the convoluted tubule, the bichromates, corrosive sublimate and the uranium salts. For the rabbit, they considered 2 mgm. per kilogram as the minimum fatal dose. The injury induced was essentially one of the convoluted tubules and to a less extent of the cells of the loops of Henle. When much larger doses, 20 mgm. per kilogram were employed, the injury was more diffuse, but remained tubular in character. In all of the animals the glomeruli appeared normal.

In a final series of experiments 1 mgm. per kilogram of uranium was given to rabbits. Such animals developed an acute nephritis followed by recovery.

The functional side of this investigation was concerned with a study of the diuretic value of Ringer's solution and a 5 per cent solution of caffein sodium-benzoate, in the hope of ascertaining the localization of the diuretic effect of these substances; whether in the glomeruli or at the tubular epithelium.

Normal animals were used as controls and in such animals a free diuresis was obtained from the different types of diuretic solutions.

In the uranium animals, if the animal was not responsive to a secretory diuretic as caffein, it was also not responsive to a mechanical diuretic as a sodium chloride solution of Ringer's solution. The nephritic kidney responded slower to all forms of diuretics than did the normal kidney and the urine formed by the nephritic animals showed a decrease in the elimination of sodium chloride.

These experiments would tend to emphasize the importance of the epithelial element of the kidney as a functional unit, for according to the anatomical observations of these investigators uranium spares the glomeruli but severely injuries the epithelium.

The foregoing review of various histological and functional studies of the nephrotoxic action of uranium would indicate that the substance is more of an epithelial than a vascular poison, but that this action is variable in its severity in animals of different species and also in animals of the same species. Some years ago an observation was made by MacNider (42) which may have some bearing on the variation in the toxic effect of uranium. After working with a large number of dogs in which a constant quantity of the poison was given subcutaneously per kilogram it became evident that the age of the animal in some measure determined not only the local nephrotoxic action but influenced the general uranium intoxication. Both albumin and glucose as well as the ketone bodies appeared earlier and in larger amounts in the urine of adult and old animals than they did in the urine of puppies, even though the puppies on account of their weight might have received a larger total dosage of the poison than had the older animals. In a later paper (43) these studies were continued and the further observation was made that the toxic effect of uranium for the kidney depended upon the ability of the substance to induce a tissue acidosis. This metabolic disturbance in the older animals was more severe than in the young animals and in the former group of animals the epithelial degeneration in the kidney was more extensive and complete. In addition, the functional response of the kidney of animals of different age periods as indicated by the use of various diuretic solutions, showed a parallel with the degree of acid intoxication and with the severity of the histological changes which had developed in the renal epithelium.

Later studies by Karsner and Reiman (44) and by Karsner, Reiman and Brooks (45), (46), (47) have shown that uranium is eliminated both by the liver and the kidneys. They consider the injuries to these organs largely due to the action of uranium as such. They have however observed the constant presence of an acid intoxication in dogs poisoned

by this metal and have noted that the intoxication was more severe in old animals then in puppies. The alkali reserve of the blood was reduced at the time of the appearance of albumin in the urine.

MacNider (48) in a paper of some years ago considered the toxicity of uranium to be associated with the power of the metal to lead to the formation of organic acids, for the experiments showed the ability of a solution of sodium carbonate to lessen the toxicity of uranium which was associated with the delayed formation in lessened amounts of such acid bodies and was not dependent upon the ability of the carbonate to detoxicate a solution of uranium. It was furthermore shown that, when protected and unprotected animals were anesthetized, the protected animals were more responsive to diuretic solutions than the control animals which had not received intravenously a solution of sodium carbonate. The protected animals showed much less evidence of epithelial injury.

Similar observations (49), (50) have been made concerning the ability of an alkali to influence the amount and distribution of stainable lipoid material in normal animals, animals acutely nephropathic from uranium, and in naturally nephropathic animals both before and after the use of a general anesthetic (51), (52), (53). The use of such a solution decreases the degree of reduction in the reserve alkali of the blood from uranium and from an anesthetic and at the same time decreases the amount of stainable lipoid material in the cells of the loops of Henle and in the convoluted tubule cells. When such a change in the physico-chemical state of the blood and in the histology of the kidney has been induced, both the toxicity of uranium and of the general anesthetics is decreased for the kidney. More recently a study (54) has been made of renal function and the associated disturbance in the acid-base equilibrium of the blood in the experimental acute nephritis from uranium and bichloride of mercury and when such an acute injury was superimposed upon a chronic naturally acquired kidney injury by the use of uranium. These studies have shown a very rapid reduction in the reserve alkali of the blood from both uranium and bichloride which is associated with an acute degeneration of the convoluted tubule epithelium. There is a rapid reduction in the elimination of phenol-sulphonephthalein and a retention of blood urea and creatinin. As the animals effect a recovery from the acute nephritis there is an increase in the output of the dye and a decrease in the retention of urea and creatinin which do not return to the normal. In animals which show functional improvement there is a restoration to the normal in the sub-

acid-base equilibrium of the blood. The repair changes in the kidney have consisted in the replacement in the convoluted tubules of a flattened, less specialized type of cell and a beginning fibrosis of the glomeruli.

The naturally nephropathic animals in which an acute injury was induced by uranium or bichloride of mercury have shown a more rapid and extensive epithelial destruction, a more marked functional disturbance, and an inability of the kidney to institute such changes of repair that would enable the animals to survive.

In 1917 Goto (55) made a study of the acidosis, blood urea and plasma chlorides in dogs with an acute uranium nephritis and of the protective action of sodium bicarbonate. He was able to demonstrate the existence of an acid intoxication in such animals and found associated with this disturbance the appearance of albumin and casts in the urine and a retention of blood urea and chlorides. When such animals were given sodium bicarbonate by mouth in the dose of 1 gram per kilogram there occurred a decrease in the degree of acidosis, of albumin and casts in the urine, and a less marked retention of blood urea and plasma chlorides. The severity of the renal pathology was definitely diminished in the group of animals that were given the alkali by mouth.

In a later paper Goto (56) continued these studies but employed potassium dichromate, arsenic, diphtheria toxin and cantharidin as nephrotoxic agents. In all of these intoxications he obtained a reduction in the reserve alkali of the blood. The use of a solution of sodium bicarbonate decreased this disturbance and tended to restore the normal equilibrium. The histological studies of the kidneys failed to show but little difference in the severity of the injury in animals that had the sodium bicarbonate protection from those animals employed as controls which did not have it.

Very recently Nuzum and Rothschild (57) have induced an acute nephritis in the rabbit with uranium and have made extensive studies of the development of the acid intoxication and the disturbed renal function. Acetone and diacetic acid appeared very early in the urine of such animals but was preceded by a disturbance in the acid-base equilibrium of the blood which was the first evidence of a departure from the normal during such intoxications. This change in equilibrium was not due to a retention of ketone bodies. Several of the animals after having received as much as 40 mgm. of uranium failed to show these bodies in the urine, while the volume per cent of carbon dioxide in the blood might be as low as 25.3 to 33 and the reserve alkali of the blood as low as 7.6.

They conclude that the relative degree of acidosis as determined by the carbon dioxide combining power of the blood and the reserve alkali readings, coincide pathologically with the extent of cell destruction in the kidney.

From the foregoing review of the experimental work which has concerned itself with the development and significance of an acid intoxication in various forms of acute experimental nephritis, it would appear just to assume that certainly in an acute uranium nephritis the injury to the kidney is in large measure due to the metabolic disturbance effected by the uranium and characterized by the development of an acid intoxication and that such an intoxication is not primarily a pretention phenomenon.

The experimental work commenced by Schlayer and his associates in 1907 which was concerned with the production of an acute nephritis by various nephrotoxic agents with a study of the functional response of the kidney in such pathological states, has been not only productive of new information in this field of experimentation, but it has stimulated interest as has been shown by many researches on renal function both in the normal and pathological kidney.

Schlayer and Hedinger (58), Schlayer, Hedinger and Takayasu (59), Takayasu (60) and later Mosenthal and Schlayer (61) were primarily concerned with an attempt to correlate the functional and anatomical changes developing in the kidney rendered acutely nephritic by certain nephrotoxic agents which they considered either vascular or epithelial in their action. For this purpose they used in the rabbit, diphtheria toxin, cantharidin and arsenic to induce a vascular type of injury, and potassium dichromate and bichloride of mercury to effect a tubular injury. In their later work Mosenthal and Schlayer used uranium. The vascular response of the kidney was investigated in these two types of nephritis. They postulated that the vascular response should differ in the tubular and vascular types of injury and that this could be ascertained by the use of stimuli causing a contraction or dilatation of renal vessels as measured by an oncometer.

For a central effect in constricting the renal vessels they used tobacco smoke in the nostrils of the animals, a transitory suffocation, or adrenalin. Either a hypertonic solution of sodium chloride or a solution of caffein was used to induce a dilatation of the renal vessels.

In normal control animals the use of adrenalin, tobacco smoke or asphyxiation induced a rise in general blood pressure, a constriction of the renal vessels and a decrease in urine formation. Dilator agents

such as hypertonic sodium chloride solution and caffein induced little or no change in general blood pressure but an increase in kidney volume and an increase in urine formation.

When these methods were employed in their tubular type of nephritis from bichloride of mercury or a chromate, they found that the vascular response of the kidney in the early stages of the injury did not vary from the normal, but that urine formation was greater than in the normal animal and that the power of the renal vessels to contract and dilate was greater. There was no histological evidence of vascular injury but marked tubular injury. In the late stages of such a tubular injury the constrictor reponse of the vessels from central and peripherally acting stimuli was normal, but the power of the renal vessels to dilate from caffein or hypertonic salt solution was decreased or lost and diuresis did not occur. In this stage of the nephritis there was a tubular injury plus a functional glomerular disturbance.

The vascular type of nephritis was induced by arsenic, cantharidin, and diphtheria toxin, and similar methods employed for the functional study of the vascular response. Schlayer and his associates observed when such renal poisons were used that within a few hours the effect of both dilator and constrictor stimuli on the renal vessels had decreased and diuresis was diminished. Finally the response was lost and an anuria was established. Their account of the pathology of the kidney in the vascular types of nephritis is not comprehensive. There was not much epithelial injury.

These investigations would clearly differentiate between a vascular and epithelial type of injury on the basis of the functional response of the glomeruli to certain stimuli and would support in no indefinite fashion the contention that the glomeruli are the main functional units in the kidney.

According to these studies, with a marked tubular injury, a normal or exaggerated vascular response, and with no structural changes in the glomeruli they obtain a free diuretic effect from caffein and a sodium chloride solution. In the vascular type of nephritis with little histological evidence of vascular or epithelial injury but in which there is a lack of vascular response, urine formation decreases or is arrested.

In a later paper by Schlayer and Takayasu (62) they came to the further conclusion that the glomeruli may be functionally deranged even though they show no histological evidence of injury. They were inclined to discard histological evidence, and determine glomerular function by estimating the contraction and dilatation of the renal vessels and the associated degree of urine formation.

The experimental work of Schlayer with its newness of method and interpretation was at once followed by numerous investigations such as those of Theohari and Giurea (63) that in large measure confirmed Schlayer's work and by those of V. Monakow (64) who doubted if Schlayer's functional test was better than the histological evidence of injury to the glomeruli, for according to his reasoning the capsular epithelium might perform its function even though the capillaries of the glomeruli did not respond to stimuli.

Soon after these studies investigations appeared by Pearce, Pearce, Hill and Eisenbrey (65), and MacNider (66), (67), (68) who approached several of the problems of acute nephritis by methods similar to those employed by Schlayer and his co-workers.

The work of Pearce and his associates was in general a repetition of Schlayer's experiments in a study of the vascular response of the kidney in acute injuries, and in addition observations on nitrogen elimination. They used in the dog potassium dichromate, uranium nitrate and bichloride of mercury to induce a tubular type of nephritis and for the vascular type arsenic and cantharidin. They obtained an early epithelial injury from the tubular poisons with no evidence of vascular injury except the increased ability of the renal vessels to respond to dilator and constrictor impulses with an increase in urine formation.

The study of the vascular poisons showed that at the time of the development of only slight tubular injury there occurred an anuria which was associated with a decrease in the vascular response of the kidney. Pearce did not consider the nephritis induced by these substances to be pure in type, and states that the tubular lesions from chromium, uranium and bichloride of mercury pass into a stage resembling the vascular type if not identical with it. He describes two forms of tubular nephritis; an anuric type associated with a gastroenteritis in which the vascular response of the kidney shows a decreased ability of the vessels to dilate and a decrease in urine formation. In the second type the animals are polyuric until anesthetized. The renal vessels after the development of the anesthesia are responsive to stimuli yet no diuretic effect is obtained from caffein and a solution of sodium chloride. He assumes that in the polyuric type of animal which rapidly becomes anuric a damage has taken place to the permeability of the glomeruli. He does not discuss the epithelial damage associated with such an alteration in functional response.

In their studies of nitrogen elimination in the two types of nephritis, Pearce, Hill and Eisenbrey observed a decrease in the output of nitrogen

by animals with a tubular type of injury while in the vascular type as represented by arsenic the output was not only not decreased but considerably increased.

The experiments conducted by MacNider were carried out on dogs both acutely nephritic from uranium and with a chronic naturally acquired nephritis.

In the first three investigations previously referred to, animals were rendered acutely nephritc by uranium, anesthetized by morphine-ether or by Gréhant's anesthetic and subjected to an experimental technique similar to that employed by Schlayer and later by Pearce. Adrenalin was used to test the power of contraction of the renal vessels while a solution of caffein and hypertonic sodium chloride were used to effect a peripheral vasodilatation. The caffein solution is not only of value for this purpose but on account of much evidence that it stimulates renal epithelium it is of value in ascertaining the functional response of this tissue in the kidney. In addition to these substances the following solutions supposed to be of diuretic value were employed. Theobromine, theocin, digitalin, isotonic sodium chloride solution and a 20 per cent solution of glucose in a 0.9 per cent sodium chloride solution. General blood pressure determinations were made from the carotid artery and the changes in renal volume were either graphically recorded or measured by a water manometer connected with the oncometer. The results obtained in these three series of experiments have been uniform, and have in large measure depended upon the type of anesthetic substance used for the experiment and to a less extent upon the age of the animal.

After these acutely nephritic animals had developed a state of anesthesia they have grouped themselves functionally into those animals which remain diuretic and responsive to the diuretic solutions and into a group that rather rapidly become anuric and fail to respond to the same diuretic substances. In general the diuretic group of animals has been represented by those dogs anesthetized by morphine-ether and the puppies which were anesthetized by Gréhant's anesthetic, while the anuric group was represented by a few of the old animals receiving a morphine-ether anesthesia and the adult and old animals anesthetized by Gréhant's mixture.

In these two groups of animals there has been no marked difference in the carotid blood pressure at the commencement of the experiments or in the ability of the solutions to transitorily raise the pressure. The animals anesthetized by Gréhant's anesthetic, the main anesthetic ingredient of which is chloroform, tend to have a lower general blood

pressure. Furthermore, and of much importance in the light of the experiments of Schlayer and Pearce, the ability of the renal vessels to dilate and undergo constriction has shown no difference in the two groups which differed so widely in their response to such changes in so far as urine formation was concerned. In certain of the anuric group of animals these responses were even greater than in the diuretic animals, yet in such animals with the glomerular response either normal or exaggerated there was no functional response on the part of the kidney from any of the varied diuretic solutions.

Prior to the death of the anesthetized animals the kidneys were removed for histological study. These studies have shown that those acutely nephritic animals which were anesthetized by morphine-ether or by Gréhant's anesthetic and remained diuretic and responsive to diuretic solutions to have but slight evidence of either glomerular or tubular injury. The glomerular vessels were packed with blood. There was usually no subcapsular exudate and no hemorrhage.

The tubular epithelium and especially that of convoluted tubules showed some swelling and vacuolation but not necrosis. Stainable lipoid material was present in the cells of the loops of Henle and to a less extent in the convoluted tubule cells.

The anuric group of animals which were mainly represented by those animals anesthetized by Gréhant's anesthetic have shown a glomerular pathology identical to the diuretic group, but a marked difference in the degree and extent of the epithelial injury. In the anuric group the convoluted tubule epithelium has shown a severe swelling, vacuolation and necrosis with fragmentation of the nuclei. The amount of stainable lipoid material in the cells of the loops of Henle and in the convoluted tubule cells has been greatly increased over that observed in the diuretic group of animals anesthetized by morphine-ether.

These experiments indicate that uranium injures the glomeruli to a slight extent without interfering with the ability of the glomerular capillaries to undergo constriction and dilatation, but that it exerts its main toxic effect upon the tubular epithelium. When an anesthetic such as Gréhant's is employed as was also used by Pearce, the tubular injury becomes greatly exaggerated and the animals become anuric. This acutely anuric group which follows an anesthesia was first noted by Pearce. In such animals the carotid blood pressure may be raised, and the vascular response of the kidney may be increased or decreased to a degree even greater than that obtainable in a diuretic animal without establishing a flow of urine. The anuria is not dependent upon a lack

of functional response on the part of the glomeruli and may not be due to any decrease in the permeability of the glomerular vessels; experiments have not been undertaken to ascertain this possibility. In all of the experiments in which the animals became anuric the lost function on the part of the kidney has been associated with the degree of injury which uranium plus the anesthetic effects in the epithelial mechanism of the kidney.

The second group of experiments (69), (70), (71) were conducted with naturally nephropathic animals. The first series of these experiments were essentially anatomical in character. Dogs were found to develop, likely as a result of chronic skin infections, a glomerulonephritis, both capsular and intracapillary, with but slight evidence of tubular injury. Such animals were freely diuretic with a urine that contained both albumin and casts and rarely glucose. When such animals were anesthetized by Gréhant's anesthetic or rendered acutely nephritic by uranium and then anesthetized, they became anuric without any acute injury having been superimposed on the chronic naturally acquired glomerular pathology other than an acute congestion of the glomerular vessels. The tubular epithelium became vacuolated and necrotic. The functional response of such kidneys was studied by a technique identical to that described for the acutely nephritic groups of animals.

In this last group of animals with a chronic nephritis in which acute changes had been superimposed, the determining factor in the ability of the animals to form urine and to respond to diuretic solutions was the degree of preservation of the renal epithelium and not the degree of vascular response on the part of the renal vessels.

The experimental work of Schlayer, Pearce and their associates not only served to stimulate work concerning the selective affinity of various nephrotoxic agents but investigations were undertaken to determine the value of various diuretic substances, and of the ability of the acutely injured kidney to eliminate various end products of metabolism and certain dyes which leave the organism in large part by the kidney.

Castaigne and Rathery (72) working with dogs with an acute nephritis noted a decreased chloride excretion as compared with normal dogs. If a salt solution was injected into the renal artery a retention was not observed. They concluded the decreased salt excretion was due to a tissue retention and not to a decrease in the permeability of the kidney for salt.

Pohl (73) in his work on animals that had received small doses of uranium observed an initial polyuria as did Chittenden and Lambert, and

furthermore determined the output of albumin. There occurred in such animals a high excretion of urea, total nitrogen, potassium and sodium chloride. The animals finally died of inanition. The kidneys showed a destruction of tubules.

In 1908 Christian (74) working with Smith made a study of cast formation in acutely nephritic animals and came to the conclusion that all casts were composed of material primarily coming from the tubular epithelium. Granular casts were relatively young casts while the hyaline variety were older.

Weber (75) in his study of animals with an early tubular injury noted an increase in the elimination of water and a greater response from diuretics than occurred in normal animals. Such an observation might be explained on the basis of the injured tubule being unable to reabsorb fluid formed at the glomerulus.

Siegel (76) working with dogs with a uranium intoxication demonstrated a decrease in the output of nitrogenous bodies and observed that when this occurred there was usually an increase in fecal nitrogen, indicating perhaps a compensatory mechanism for nitrogen elimination on the part of the intestine.

An attempt to modify the uranium injury was made by Tria (77), who induced an acute nephritis with uranium in dogs, and tried the curative value of obtaining blood serum from the renal vein of goats and injecting the nephritic animals. Frank (78) at about this period made an extensive study of the toxic effect of many of the heavy metals but was mainly concerned with the glycosuria produced by these substances.

In 1911 Eisenbrey (79) published one of the earlier and most important papers on the elimination of phenolsulphonephthalein in normal control animals and in animals with an acute nephritis from uranium, potassium, dichromate, cantharidin, arsenic, diphtheria toxin, Crotalus venom, nephrotoxic immune serum and hemolytic immune serum. He extended his observations to animals with a mechanical injury to the kidney induced by occluding with a screw clamp both renal arteries for periods of from 15 to 60 minutes. Similar observations were finally conducted in three naturally nephritic animals. In the normal control dogs the dye appeared in the urine on an average in five minutes, and had a total output in two hours of 72.33 per cent. In the naturally nephritic animals the two hour output varied from 41.2 to 66.9 per cent.

In the acute nephritis induced by uranium, potassium dichromate, cantharidin, diphtheria toxin, arsenic and Crotalus venom, reduction in elimination was marked. From the uranium injury there was a transi-

tory increase in elimination followed by a progressive decrease. In the animals with a chromate nephritis there was no initial increase but a very rapid reduction in output. With arsenic there was a temporary slight reduction followed by no change until the fifth day when the output of the dye was markedly diminished. In the animals with renal injuries induced by the nephrotoxic and hemolytic immune sera the elimination of the dye was increased rather than reduced.

Folin, Karsner and Dennis (80) produced acute nephritis in the cat by the use of uranium, potassium dichromate, and cantharidin. They considered uranium to involve both tubules and glomeruli, though the injury was more marked in the epithelium. The uranium induced a marked blood nitrogen retention while with the chromates that affected more exclusively the tubules the retention was less marked. Cantharidin which injured the glomeruli and the tubules to a degree greater than the chromates caused an early and marked nitrogen retention which persisted.

The decreased function not only in terms of water secretion but in terms of elimination of dyes and various organic substances in solution in the epithelial injuries of the kidney has been attributed to the swollen epithelium interfering with the circulation through the kidney. In this connection the perfusion experiments of Ghoreyeb (81) are of interest. He conducted perfusion experiments with a double strength Adler's solution in kidneys that had been rendered nephritic by a variety of substances including uranium and came to the conclusion that there was less mechanical obstruction to the circulation in those kidneys injured by epithelial poisons which induce necrosis of the epithelium than those in which the glomerular injury is dominant with an associated swelling of the epithelium.

Following these scattered observations concerning the functional response of the kidney in an acute uranium nephritis, Christian and his associates undertook a long series of the most thorough studies which dealt with urine formation, the value of various diuretics, and the ability of the kidney to eliminate various substances not only in animals anesthetized and observed over short periods, but in animals attempting a recovery from such nephritic processes which extended over weeks. A report of many of these experiments was made by Christian (82) in 1913. In this series he studied the effect on urine formation and nitrogen retention of caffein, theocin, spartein sulphate and diuretin. These substances were given by mouth and intravenously to twenty rabbits in the same proportion per kilogram as when given to man.

Diuretin given twice a day induced no distinct increase in urine formation or in nonprotein nitrogen or urea nitrogen retention. In animals with a severe uranium nephritis the diuretic value of diuretin varied but the nitrogen retention steadily increased as the animals approached death.

Walker and Dawson (83), working along the same line of research, gave rabbits with a uranium intoxication daily doses of theocin, caffein, potassium acetate and spartein sulphate. In a severe nephritis they found the use of these drugs to actually shorten the lives of the animals. In less severe injuries theobromine sodium-salicylate appeared to have a beneficial effect by increasing the output of urine unless it was repeated often enough to cause renal fatigue.

In anesthetized rabbits with a uranium nephritis Fitz (84) found the use of theobromine sodium-salicylate and theocin to increase the flow of urine and the elimination of sodium chloride and nitrogen. Their use did not increase the elimination of phenolsulphonephthalein. In animals with a chromate nephritis there was no increase in the output of nitrogen and a rapidly diminishing elimination of phenolsulphonephthalein.

Christian (85) also studied the elimination of this dye in uranium animals and failed to observe any constant effect of diuretics. Smille (86) working with rabbits with a uranium nephritis found that a stage of the intoxication was reached as measured by 100 mgm. of nonprotein nitrogen per 100 cc. of blood in which the kidneys were difficultly permeable to potassium salts. In such states the potassium was toxic and caused the death of the animals while animals with a normal renal function found no difficulty in tolerating a similar amount of a potassium salt.

The later research of Mosenthal (87) was solely concerned with the elimination of nitrogen by uranium dogs. He found the elimination variable, either increased or decreased, and suggested that the increase was due to an increased protein catalysis with a kidney functionally competent, while the decrease in elimination occurred when the kidney became so impaired as to be functionally incompetent for this substance.

Closely related to these experiments are those of Kraus (88) who studied the elimination of creatinin, uric acid and chlorides in dogs with a uranium nephritis, and also investigated the effect of creatinin injections in such animals. The experiments showed that in an acute uranium injury creatinin excretion is decreased while the uric acid and chlorides are increased. In a subacute uranium injury all three substances are decreased. The effect of creatinin injections in the acute stage of the nephritis caused creatinin, uric acid, chlorides and water to be excreted in diminished amounts leading to death of the animals.

Fitz (89) continued his functional studies in uranium nephritis by contrasting amylase and phenolsulphonephthalein elimination with the degree of nonprotein nitrogen retention. He found that amylase in the urine and nonprotein nitrogen of the blood varied from the normal during the course of a uranium intoxication and that as the injury heals they return to the normal. The amylase excretion was less accurate than the phenolsulphonephthalein elimination. Later than this Quinby and Fitz (90) induced a unilateral uranium injury by injecting the poison into the renal artery and comparing the functional response of the normal with the injured kidney. In one group of animals the kidney was hyperpermeable to water, chlorides, nitrogen and more rarely to phenolsulphonephthalein and indigo carmine, while in a second group of animals with a well marked nephritis there was decreased function and retention. Phenolsulphonephthalein elimination and nitrogen retention were the most satisfactory tests. In such experiments the renal injury must be more of a corrosion than a true nephritis.

Functional studies similar to the preceding researches were carried on at this time by Frothingham, Fritz, Folin and Dennis (91) in which the elimination of phenolsulphonephthalein was contrasted with the retention of urea nitrogen and nonprotein nitrogen. The elimination of the dye was found to drop very abruptly with the establishment of the nephritis while the blood nitrogen slowly increased. The elimination of the phenolsulphonephthalein should be looked upon as expressing the immediate functional depression of the kidney while the retention or blood nitrogen indicates the result of the past and the present interference with renal function.

A functional study very similar to this was undertaken by Karsner and Dennis (92). A variety of nephrotoxic agents were employed. In these studies they obtained only a slight nitrogen retention from the use of arsenic and diphtheria toxin but from the tartrates which induced an early and marked epithelial injury the retention was marked. The elimination of phenolsulphonephthalein showed a parallel with the retention, and in cases of recovery from the nephritis the output of the dye first increased with a later decrease in the retention of nitrogen.

In a second series of experiments these investigators (93) studied nitrogen retention in the cat following repeated small injections of both uranium and diphtheria toxin which would induce a subacute or chronic nephritis that would progress to a fatal termination. The uranium animals showed a steady increase in the retention of nonprotein nitrogen while the animals receiving the toxin showed only a temporary and slight retention.

With the fact established through numerous investigations that in a uranium nephritis there occurs a nitrogen retention, Watanabe, Oliver and Addis (94) used urea injections as a functional test in such intoxications and found a definite relationship to exist between the injury to the kidney and its function as indicated by the ratio between urea in the urine and blood.

Underhill and Kapsinow (95) have recently studied in the same type of nephritis the relationship between creatinin and nonprotein nitrogen retention and blood concentration. They found that the greater the retention the more dilute the blood became.

The study of blood concentration and blood volume in experimental nephritis was first undertaken at about the same time by Boycott (96), (97), (98) and Chisolm (99), (100), (101) and by Boycott and Douglas (102). Boycott's investigations consisted in a study of blood volume and its concentration in normal and nephritic animals. Rabbits were given uranium, and later 48 cc. per kilogram of normal saline solution or Ringer's solution intraperitoneally. The nephritic animals became edematous while the normal animals failed to show edema or collections of fluid in the serous cavities.

The changes in blood concentration were ascertanied by measuring the concentration of hemoglobin. The nephritic animals showed tissue fluids to contain more protein and more chlorides than the normal animals.

Chisolm in his work used potassium dichromate in addition to uranium and employed rats as experimental animals. These poisons induced extensive tubular necrosis and associated with this renal pathology in animals that were given Ringer's solution there was an accumulation of fluid in the kidneys, muscle and subcutaneous tissues.

Somewhat later than these studies, Tribe and Barcroft (103), Tribe, Hopkins and Barcroft (104) and Tribe, Harvey and Barcroft (105) investigated certain vascular and metabolic conditions in normal rabbits and in rabbits rendered nephritic by uranium acetate and diphtheria toxin. They found in the uranium animals the glomeruli shrunken, the subcapsular spaces prominent, with extensive necrosis of the tubular epithelium. The blood flow through such kidneys was faster than normal but oxygen consumption was reduced.

In the diphtheria toxin nephritis the glomeruli were congested with also an epithelial necrosis. Such animals had a low systolic blood pressure, the flow of blood through the kidney was reduced, and oxygen consumption was greatly interfered with. The experi-

ments would indicate that the condition of the glomeruli has more to do with the rate of flow of blood through the kidney than has the swollen and necrotic epithelium.

CONCLUSIONS CONCERNING ACUTE URANIUM NEPHRITIS. 1. Uranium induces an initial polyuria followed by a decrease in urine formation and finally an anuria. The essential and dominant pathology in the kidney is an injury to the convoluted tubule epithelium, with at first a congestion and later degenerative changes in the glomeruli. Oxygen consumption on the part of such a kidney is reduced.

2. In the early stages of such an injury the use of secretory and saline diuretics is followed by some increase in urine formation and an increase in the elimination of chlorides, phenolsulphonephthalein, urea and nonprotein nitrogen. Later in the nephritis such diuretics are ineffectual and when used in recovery experiments may even be harmful by decreasing the length of the life of the animals.

3. Whether or not the inability of the kidney in a uranium nephritis to respond to such diuretic substances is due to a lack of vascular response on the part of the glomeruli or to the renal epithelial cells being so injured that they fail to respond to such stimuli is an open question for solution.

4. The use of uranium induces an acid intoxication which can not be solely considered as a retention phenomenon.

5. The functional depression of the kidney in a uranium nephritis is first shown by a decrease in the elimination of phenolsulphonephthalein and by a retention of chlorides, blood urea, nonprotein nitrogen, creatinin and uric acid.

II. ACUTE CHROMATE NEPHRITIS. Gergens (106) in 1876 first made the observation of the toxic effect of the salts of chromic acid for the kidney. Since this time these substances have been extensively employed to induce an acute nephritis and with less success to induce a chronic injury.

Hellin and Spiro (107) were amongst the early investigators who used the chromates for not only an anatomical study but for functional observations on the kidney. They found that caffein induced a diuretic effect in animals nephritic both from chromates and aloin but failed to do so following the use of cantharidin. They assumed that cantharidin acted through the glomeruli and that no effect was obtained from cantharidin on account of the glomerular injury while the chromates and aloin which were tubular poisons spared the glomeruli and permitted a caffein diuresis.

Later than this Bordier and Frenkel (108) induced a unilateral kidney injury by injecting a solution of chromate into one renal artery and compared the urine secreted by the two kidneys. The urine from the injured organ was smaller in amount and the concentration lower. The elimination of chlorides was high at first but later returned to the normal.

Weber (109) in his work with the chromates observed, as is the case with so many nephrotoxic agents, an increase in urine formation at the commencement of the nephritis. This was followed by an anuria which was uninfluenced by theocin. Ophüls (110) in his attempt to induce a chronic type of nephritis in the dog gave the animals potassium dichromate in milk. The acute renal injury consisted primarily in the necrosis and detachment of renal epithelium.

In 1908 Pearce and Sawyer (111) became interested in the nephrotoxic substances developing in the blood serum of animals nephritic from potassium dichromate and uranium. They found as did Lindemann (112) that the serum from such animals when injected into normal dogs induced a renal injury associated with the appearance of albumin and casts in the urine. The serum from normal dogs failed to have this action. In these experiments the injury was largely tubular.

From the anatomical studies previously referred to and from the anatomical and functional findings of Green (113), Pearce, Hill and Eisenbrey, Schlayer and his associates, Ghoreyeb and MacNider (114), there would appear to be but little doubt that of all the nephrotoxic agents employed for experimental purposes the chromates exert the most selective affinity for the tubular epithelium.

In 1912 Rowntree and Geraghty (115) conducted an extensive series of experiments on animals and supported their anatomical findings with observations on the elimination of phenolsulphonephthalein. When renal injuries were induced by tubular poisons such as the chromates there was an early and marked reduction in the elimination of the dye while in a nephritis induced by vascular poisons as arsenic the reduction in the output was delayed, until such poisons secondarily involved the glomeruli. This study not only points out the affinity of different nephrotoxic agents for the vascular or epithelial elements of the kidney but it indicates that the point of elimination in the mammalian kidney for phenolsulphonephthalein is certainly largely the convoluted tubule epithelium. This observation has been confirmed in a very definite fashion by the recent observation of Marshall and Vickers (116).

In 1914 Fitz (117) anesthetized rabbits with a chromate nephritis with urethane and studied the effect of theocin and theobromine sodium-salicylate on the flow of urine and the elimination of chlorides and nitrogen. There was no increase in secretion following such substances and the elimination of phenolsulphonephthalein was sharply reduced. O'Hare (118) conducted very similar experiments in recovery animals from a chromate nephritis. He found that in a moderately severe nephritis the use of diuretics shortened the lives of animals as was the case when uranium was used, while in a mild nephritis the use of the diuretics appeared to prolong life. Such substances had no effect on the elimination of phenolsulphonephthalein, nitrogen and water. Finally the work of Levene and Kristeller (119) and of Lefmann (120) has established the tubular character of the chromate injury and the inability of such a kidney to eliminate creatinin.

Mosenthal and Schlayer (121) studied the effect of fatiguing the kidney nephritic from a chromate by the use of a sodium chloride solution. They were able to effect such a condition as was indicated by a decrease in renal function, and furthermore found that if caffein was used prior to the salt solution the state of fatigue was more readily induced.

The most recent study of the chromate nephritis has been made by Goto (122). In such injuries he obtained an acid intoxication with a retention of plasma chlorides, blood urea and nonprotein nitrogen. To a group of such animals he administered 2 grams of sodium bicarbonate per kilogram in the hope of obtaining the beneficial results previously observed in uranium nephritis. Histological studies of the kidneys of such animals failed to show any beneficial effect from the use of the alkali.

Conclusions concerning acute chromate nephritis. 1. The chromates induce a renal injury which is primarily tubular and effects in particular the epithelium of the convoluted tubules. Late in such a nephritis the glomeruli participate in the injury.

2. Secretory diuretics such as caffein, theocin, and theobromine sodium-salicylate are of no value and may be injurious.

3. The chromates lead to a rapid reduction in urine formation, an inability of the kidney to eliminate phenolsulphonephthalein, and a retention of chlorides, blood urea, nonprotein nitrogen and creatinin.

4. The chromate nephritis is associated with the development of an acid intoxication which is likely a retention phenomenon and which is not favorably influenced by the use of an alkali.

III. ACUTE BICHLORIDE OF MERCURY NEPHRITIS. The ability of certain of salts of mercury to increase urine formation in both the normal and pathological animal has been recognized for many years. This observation has been considered from an experimental point of view by Cohnstein (123), Vejux-Tyrode (124) and later by MacNider. The mechanism of the action is not understood. Ruschaupt (125) obtained a similar effect in animals from the chromates when they were employed in very small quantities.

In 1860 Pavy (126) induced an experimental nephritis with mercury. He considered the Malpighian bodies to be the seat of an injury and also noted the necrosis of the tubular epithelium which contained calcium phosphate. Von Mehring (127) considered the toxic action of the bichloride for the kidney and intestine to be due to a vasomotor paralysis and this blood vascular effect was also accepted by both Heineke (128) and by Kaufmann (129), (130), who considered that mercury in the blood had a thromboplastic action and that the damage to the kidney depended upon the formation of thrombi with the production of infarcts. From my experiments I feel certain that in mercuric chloride poisoning the clotting time of the blood is greatly diminished. This is an observation which has not been determined by actual measurements.

In 1895 Leutert (131) entered into a detailed discussion of the processes of calcification in bichloride poisoning and at about the same time Hellin and Spiro (132) after inducing a bichloride nephritis studied the effect of caffein as a diuretic. There was an increase in urine formation over that which could be obtained in normal animals.

Harnack and Kustermann (133) have described in detail the fatty changes in the epithelium of the cat kidney in bichloride poisoning and later in the injury the connective tissue hyperplasia.

One of the most important of the early investigations of the bichloride nephritis was made by Karvonen (134). The studies were made in both rabbits and dogs. He observed an infiltration around the blood vessels of a leucocytic like cell which became transformed into fibroblasts with the formation of connective tissue. In the acute injury there was a congestion of the glomeruli and an exudate inte the subcapsular spaces, while the glomerular endothelium and capsular

epithelium showed desquamation. Areas of necrosis were found in the tubules.

At a later date Aschoff (135) not only noted the changes in urine formation which had previously been made by Weber (136) but studied in detail the renal pathology which consisted in swelling, hyaline vacuolation, and necrosis of the tubular epithelium.

Lyon (137) used both cats and rabbits in his experiments on the production of an acute bichloride nephritis. The mercury was given both subcutaneously and intramuscularly. The urine showed albumin which varied from a trace to 7 grams per liter. Granular casts were present. The amount of albumin decreased on the second day and was often absent for several days prior to the death of the animals. Certain of the animals became rapidly anuric.

In these studies Lyon was unable to obtain any characteristic changes in the glomeruli. In the acute injury there was marked congestion of the capillaries but no thrombi in the vessel nor hyaline changes in the glomerular endothelium. The capsular epithelium was swollen. The tubules were irregularly involved in the injury, many appeared normal, while others showed two types of necrosis. The cells usually died in mass and resembled Weigert's coagulation necrosis. In other areas the epithelium underwent an intense swelling and granular degeneration.

Galleotti (138) and Gründwald (139) and later Pearce, Hill and Eisenbrey (140) studied in particular the value of diuretics in acute mercuric chloride intoxications. The first two investigators found that both a saline solution and caffein would increase urine formation. According to Pearce, Hill and Eisenbrey, the value of such substances depends upon whether the renal injury is of a vascular or epithelial type and characterized by an anuria. Rowntree and Geraghty (141) consider the injury to the kidney from bichloride as purely tubular and have found in such an injury a rapid reduction in the output of phenolsulphonephthalein. This observation is supported by the experiments of Burns and Cheetan (142) who used an organomercury compound of phenolsulphonephthalein to induce a renal injury on account of the affinity of both substances for renal cells. Such a substance causes the same type of renal injury as has been described by Foster (143) and by Myers and Lough (144) as developing in the human kidney in a bichloride nephritis. The work of Eisenbrey (145) and Sansum (146) agrees with that of Rowntree and Geraghty and of Burns and Cheetan concerning the affinity

of the bichloride for renal cells and its ability to rapidly reduce the elimination of phenolsulphonephthalein.

In 1917 Burmeister and McNally (147) made a detailed study of the variation in the toxicity of bichloride of mercury for experimental animals. They considered the degeneration in the liver and the kidney to be due to the mercury acting as such during absorption and elimination. They found the liver injury to vary with the duration of the intoxication and the kidney injury to vary with the amount of mercury introduced as well as with the duration of the intoxication.

About this time MacNider (148), (149) pointed out the marked susceptibility of naturally nephritic animals for bichloride of mercury and in a second paper (150) investigated the variation in the susceptibility of normal animals for this substance. These studies have shown that if an animal with a chronic naturally acquired nephritis in which the injury is largely glomerular be given by mouth bichloride of mercury, there is superimposed on the vascular injury an acute destruction of the renal epithelium characterized by swelling, vacuolation and necrosis. There rapidly develops an inability of the animals to eliminate phenolsulphonephthalein and this is soon followed by the establishment of an anuria. The anuria is uninfluenced by any type of diuretic substance.

In normal animals that have been poisoned by bichloride of mercury neither the general course of the intoxication nor the nephritis are uniform in their duration or effect. In this series of experiments depending upon the type of reaction, the animals can be divided into four groups. Falling in the first group are animals which develop an intense gastroenteritis and die in a state of shock within forty-eight hours following the use of the mercury. The kidneys are intensely congested and cyanotic. There are no glomerular hemorrhages. There is no marked change in the tubules. Apparently the early development of the shock state with its associated disturbed circulation prevents the mercury from reaching and seriously damaging the kidney.

The second group of animals have recovered from the gastroenteritis and the associated shock and have died within seven days in convulsive seizures with or without an air hunger type of breathing. Mercury could be detected in the urine which contained both albumin and casts. In such animals there was a marked reduction in the elimination of phenolsulphonephthalein, a retention of blood

urea and a reduction in the alkali reserve of the blood. The kidneys of these animals showed no marked injury to the glomeruli but a diffuse Weigert-like necrosis of the epithelium of the tubules without swelling of the cells.

The third group of animals have recovered from the gastroenteritis. After an initial reduction in the elimination of phenolsulphonephthalein and a moderate depletion in the alkali reserve of the blood, these indications of renal injury returned to the normal, the urine became free from albumin and casts with a recovery of the animals.

The fourth group of animals developed a gastroenteritis which varied in severity and from which the animals recovered. Following this period, and as late as the ninth day of the intoxication when the urine no longer contained mercury, the animals either gradually or rapidly developed an acid intoxication and became anuric. With the beginning of the development of the anuria the elimination of phenolsulphonephthalein was negative, there was a retention of blood urea, and a marked reduction in the alkali reserve of the blood. In these animals the glomeruli in general were congested and more rarely there occurred an exudate of serum and fibrin into the subcapsular spaces. The tubular epithelium and especially that of the convoluted tubules showed a most intense swelling which often occluded the lumen of the tubules. The cells were vacuolated and necrotic with fragmented nuclei.

This study may explain the variable outcome of animals rendered nephritic by bichloride of mercury. There is a group of animals which die from the shock of the gastroenteritis before sufficient time has elapsed for the development of a renal injury. A second group which show mercury in the urine, develop a necrosis of the tubular epithelium without swelling which is likely due to the mercury acting as such. The animals die in convulsions after having become anuric. A final group develops a delayed acute renal injury when the urine may have been free from mercury for some days. Such animals have a severe type of acid intoxication and an intense swelling of the renal epithelium followed by necrosis. It would appear that this delayed type of renal injury is not due to the mercury, but is in some way dependent upon the disturbance in the acid-base equilibrium of the blood which does not primarily originate as a retention phenomenon.

In a more recent study (151) of mercuric chloride intoxications in dogs there has been shown to exist a liver injury with a reduction in the

reserve alkali of the blood and a decrease in blood urea prior to the development of the nephritis. Future experiments will be undertaken to ascertain the relationship which exists between an injury to the liver, the development of an acid intoxication and the secondary and delayed injury to the kidney.

CONCLUSIONS CONCERNING ACUTE MERCURIC CHLORIDE NEPHRITIS. 1. The nephrotoxic action of bichloride of mercury is variable. The dominant toxic action is on the tubular epithelium. Here there are two types of injury, a diffuse necrosis of these cells without edema, and a type in which the edema and vacuolation first develop and are later followed by necrosis. Observations have been made which would ascribe the first type of injury to the mercury acting as such, and the second type to an associated acid intoxication.

There is more histological evidence of a glomerular injury from bichloride of mercury than from either uranium or the chromates. The injury consists in an intense congestion with or without an exudate, hyaline changes in the endothelium or thrombus formation. The coagulation time of the blood appears to be decreased.

2. The value of diuretics in this type of nephritis has varied in the hands of different investigators. In some of the intoxications the use of the caffein group of diuretics and normal saline solution has been followed by an increase in urine formation, while in other series of experiments such substances have been of no diuretic value.

3. The various functional studies have shown an early polyuria followed by a rapidly developing anuria. As urine formation decreases there occurs a rapid reduction in the elimination of phenolsulphonephthalein and a retention of blood urea, nonprotein nitrogen and creatinin.

4. During the course of the nephritis from bichloride of mercury there may develop an acid intoxication the significance of which has not been determined.

IV. ACUTE CANTHARIDIN NEPHRITIS. The study of cantharidin as an acutely acting nephrotoxic agent is of particular interest on account of the diversity of opinion as to the localization of its action, and therefore when functional studies have been made of the kidney under its influence as to the functional element in the kidney which is responsible for the impaired response.

Eliaschoff (152) who was one of the earlier students of the action of cantharidin obtained marked destruction of the tubules of the

kidney in from two to three hours, while Hellin and Spiro (153) assuming that a caffein diuresis was induced through an action on the glomeruli and failing to obtain this in a cantharidin nephritis came to the conclusion that the substance was a vascular poison. About the same time Aufrecht (154) and Girmont (155) employed this poison to induce a chronic nephritis, while Browicz (156) and Cornil and Brault (157) described the acute changes in the kidney as being most marked in the glomeruli with a less important injury to the tubules. Galeotti (158) employed diuretics in the cantharidin nephritis and confirmed the earlier work of Hellin and Spiro. He found isotonic solutions of sodium chloride of little value in this type of nephritis while in the more tubular types such a solution was of value.

The most important and convincing of the earlier studies of the cantharidin renal injury was made by Welch (159). No histological observations of importance have been added to it. Welch employed a concentrated cantharidin solution in acetic ether in the dose of 0.5 to 0.1 centigram and administered this subcutaneously to rabbits and white rats. The urine from such animals contained albumin and casts, leucocytes and red blood cells. The output of urine was at first diminished and finally suppressed. At the time of such findings there occurred an intertubular leucocytic infiltration, changes in the tubules, and the most marked effect in the glomeruli. There developed between the tuft of capillaries and Bowman's membrane a wide space filled with cells and granular material often arranged in crescentric masses around the capillaries. These cells had been previously observed, and were supposed to come from proliferation and desquamation of the epithelium lining the capsule. In the rat however the capsular epitheluim was intact. Welch considers these cells which are so characteristic of the cantharidin nephritis to arise from cells in the adjacent portion of the convoluted tubules, for the mass of cells enclosed by Bowman's membrane is in direct connection with the cells in the tubules.

Argutinsky in his work on renal infarcts observed the same appearance and ascribed the presence of these tubule cells in the new location of the capsule, to be due to the pressure exerted by intertubular vessels on the tubules forcing the cells up into the capsular spaces.

Welch found the above changes not so marked in rabbits nephritic from cantharidin. There is however the same accumulation of cells

within the capsules. Opie (160) in 1912 confirmed the work of Welch as to the occurrence and origin of the capsular cell masses.

Richter and Roth (161) used cantharidin to induce a nephritis, and considered that when small doses were employed, it acted solely as a glomerular poison. There developed dilatation of the glomerular vessels with a leucocyte infiltration of the capsule. At this stage when the injury was vascular there was an increase in urine formation, while later in the nephritis when the tubules entered into the injury or early, when large doses were given the urine was diminished.

Lyon (162) made observations very similar to those of Richter and Roth in his studies of the acute and subacute nephritis from cantharidin in rabbits. He employed a solution of cantharidin in acetic ether or olive oil and made the injections intramuscularly. In cases of acute poisoning the animals survived from six hours to three days. Albumin, casts and red blood cells appeared in the urine and the severely intoxicated animals became anuric within twenty-four hours. Such animals showed enlarged kidneys with hemorrhages beneath the capsule. The cortex was swollen and congested. The glomeruli were frequently compressed by cells arranged in a mosaic fashion. Similar cells were found in the convoluted tubules. Cornil and Eliaschoff regarded these masses as lymphoid cells but the work of Welch, confirmed by Lyon and Opie, establishes their tubular origin. Finally, Lyon found the glomerular endothelium intact and the capillaries free from hyaline thrombi. According to Lyon the acute changes in the tubules consisted in a rapid necrosis of cells with an involvement first of that portion of the cell next to the lumen of the tubule. The cells of the ascending limb of Henle's loop were most often injured and next to these the convoluted tubule, cells.

Contrary to these observations is the experimental work of Bradford (163) who in a study of the selective action of renal poisons emphasizes the marked acute glomerular changes induced by cantharidin. From similar anatomical studies Aschoff (164) concludes that cantharidin induces a tubular nephritis without involving the glomeruli, while MacNider (165) would give it a place between arsenic which is so largely a vascular poison and uranium which shows a dominant action on the tubules.

Schlayer and Hedinger (166) studied the nephrotoxic action of cantharidin in their functional studies of the vascular response of the kidney in different types of nephritis. They classified cantharidin

as a vascular poison and noted the early development of vascular changes at which time the animals were not polyuric as was the case with tubular poisons such as potassium dichromate. With such a vascular injury both constrictor and dilator reactions of the glomerular vessels decreased, and with it there was a decrease in urine formation. This work was later in part confirmed by Pearce, Hill and Eisenbrey (167). In a yet later investigation Pearce (168) studied the effect of cantharidin in both dogs and rabbits by physiological methods and confirmed his earlier observation that the injury was largely vascular. His histological studies however showed degenerative changes in the tubules and in such injured cells an abundance of mitotic figures.

Eisenbrey (169) and later Rowntree and Geraghty (170) studied the elimination of phenolsulphonephthalein in cantharidin nephritis and found the output reduced. The reduction in elimination according to the latter study is not so great as from tubular poisons such as potassium dichromate and bichloride of mercury.

With the same object in view of studying the functional value of the kidney in such an injury, Folin, Karsner and Dennis (171) obtained both a glomerular and tubular injury from cantharidin and a marked nitrogen retention. They consider that the participation of the glomeruli in an experimental renal lesion increases nitrogen retention more than in those injuries which are purely tubular.

Tribe and Barcroft (172) and later Goto (173) were concerned with the metabolic disturbance occurring during a cantharidin nephritis. The former studies found oxygen consumption by the kidney reduced, the venous blood appearing redder than normal and the blood flow through the kidney increased. Goto uniformly obtained a decrease in the carbon dioxide combining power of the blood which increased with the severity of the nephritic process. The changes in the kidney were uninfluenced by the administration of sodium bicarbonate. In this connection it is interesting to note that Ellinger (174) observed that the nephritis from cantharidin was lessened when carrots were used as a diet, and that Hara (175) noted the acid urine in rabbits induced by feeding barley lessened the resistance of the kidney to cantharidin but that an alkaline urine (kara diet) protects the kidney against cantharidin.

Conclusions concerning acute cantharidin nephritis. 1. Likely with no other nephrotoxic agent is there more difference of opinion concerning the location of the renal injury than there is in

connection with cantharidin. When small doses are employed its early action shows itself mainly in the glomeruli and is characterized by acute engorgement of the capillary vessels, frequently a subcapsular exudate, but with no degenerative changes or the formation of thrombi in the capillaries. The capsular epithelium remains intact. Later in the nephritis or developing early if large doses are given there is a continuation of the congestion which shows itself in the intertubular vessels and likely through pressure explains the invasion of the subcapsular spaces by the epithelium from the proximal convoluted tubules, first described by Welch, and later by Lyon and Opie. These detached cells degenerate. With such a degree of interference with the renal circulation as now occurs and as has been demonstrated by physiological methods by Schlayer and Hedinger and by Pearce, Hill and Eisenbrey, there occurs a necrosis of the tubular epithelium. It would appear fair to state with the available evidence, that cantharidin is primarily a vascular nephrotoxic agent which through the severity of the vascular injury secondarily involves the tubular epithelium.

2. In the early stages of the cantharidin nephritis, phenolsulphonephthalein elimination is not greatly interfered with, urine formation may be even increased, and nonprotein nitrogen retention is not marked. Later, likely, when the epithelial damage has become marked, the output of phenolsulphonephthalein is reduced, there is a marked retention of nonprotein nitrogen and urine formation is decreased or an anuria is established.

3. Cantharidin intoxicated animals develop a reduction in the alkali reserve of the blood which appears to be purely a retention phenomenon secondary to the development of the nephritis.

V. THE ACUTE NEPHRITIS FROM ARSENIC AND THE TARTRATES. Considering the former frequency with which arsenic was employed for homicidal and suicidal purposes, and in recent years the prominence arsenical derivatives have assumed as therapeutic agents, it is surprising that so little work of importance has been done with this nephrotoxic agent.

Hellen and Spiro (176) considered arsenic to cause a specific paralysis of the capillaries which was most marked in the glomeruli. The subcapsular spaces became obliterated by the dilated vessels. Later the convoluted tubules became injured while the straight tubules were but slightly involved. The later study of MacNider (177) which was entirely histological agrees with this statement.

Both Schlayer and Hedinger (178) and Pearce, Hill and Eisenbrey (179) classified arsenic as a glomerular poison and in their functional studies of the response of the glomeruli to centrally and peripherally acting stimuli, found that the glomerular vessels under the influence of arsenic failed to respond at an early stage of the experiments to such stimuli. Eisenbrey (180) and also Rowntree and Geraghty (181) found that the kidney nephritic from arsenic showed a delayed elimination of phenolsulphonephthalein, which according to the latter investigators is not early in its appearance or marked as is the case with a nephrotoxic agent acting primarily on the tubules. Karsner and Dennis (182) considered arsenic to be physiologically a vascular poison but anatomically tubular in the localization of its action. They were unable to obtain but slight nitrogen retention in such an injury. More recently Pearce and Brown (183), (184) have made an exhaustive study of the nephrotoxic action for the dog kidney of arsenic and various arsenical derivatives.

An intoxication by arsenious acid leads to the production of a red kidney which drips blood. All parts of the kidney are uniformly affected, and in surviving animals the cut surface showed opaque, yellowish strictions in the cortex, the result of epithelial necrosis. Even with these changes in the tubules they considered the nephritis from arsenious acid as essentially vascular.

Salvarsan, neosalvarsan and galyl induced in general the same red type of kidney as a result of the vascular injury, but they also showed areas of tubular degeneration relating them in another respect to the kidney found when arsenious acid was used. Necrosis of the tubules in these kidneys was most marked in the inner portion of the cortex while the zone of congestion and hemorrhage was found especially in the outer half of the cortex.

The nephritis from arsacetin was found to be essentially different from the other two types of injury. The kidneys were pale, soft, and friable with but little vascular damage. The primary lesion was one of tubular necrosis. Arsenophenylglycine and atoxyl also induced a nephritis with a pale type of kidney due to the epithelial destruction but the vascular damage was more marked than that obtained from arsacetin.

The second paper of Pearce and Brown was concerned with a detailed account of the histological changes which developed in these different types of nephritides. They found the injuries to be

either very largely vascular or tubular and in each instance the reaction was of a complex character. The type of action depended entirely upon the chemical constitution of the arsenic compound which was employed to induce the nephritis.

This work is of unusual interest for it not only shows the selective affinity which a nephrotoxic agent may possess, but it makes clear that when such a substance is modified chemically, its affinity becomes altered.

Igersheimer and Rothmann (185) also studied in dogs the toxic effect of atoxyl and were able to produce characteristic renal hemorrhages with a secondary nephritis, while Luithlin (186) in his study of the toxic action of arsphenamine noted that this action was greatly increased in animals that had been subjected to vascular injuries from substances such as uranium. He concludes that the toxic action of arsphenamine is a specific action on vessels previously the seat of syphilitic disease.

Arsenious acid is certainly in small amounts a glomerular poison. The numerous hemorrhages induced by it which occur in some instances without an intense congestion would suggest an endotheliotoxic action. In prolonged intoxications or when massive quantities are used the epithelium degenerates either from the action of the arsenic as such, or from the great interference which the vascular pathology leads to in the circulation of the kidney. This factor must be of considerable importance when we consider that the kidney is encased by a relatively inelastic capsule which prevents adaptation of the tissue to a great influx of blood and favors the development of pressure on the inclosed tissue.

VI. ACUTE TARTRATE NEPHRITIS. In recent years a study of renal injuries from the tartrates has aroused much interest as a result of the initial work of Underhill who demonstrated the very selective action of these nephrotoxic agents.

Underhill and Blatherwick (187) in this and in earlier studies observed the affinity which the tartrates possessed for the cells of the convoluted tubules and the rapid decrease which such an injury effected in the elimination of phenolsulphonephthalein. Underhill, Wells and Goldschmidt (188), (189) found that in such a nephritis the glomeruli, collecting tubules and interstitial tissue showed no evidence of injury but that the convoluted tubule epithelium was completely necrosed. The secretion of urine and the nitrogenous constituents in the urine were greatly reduced. When such animals

were given urea in a solution of sodium chloride there occurred an increase in urine by some of the animals and a gradual elimination of the chloride, while the urea excretion was greatly delayed. They concluded from these experiments that water and chlorides leave the kidney through the glomerular mechanism, while urea is secreted by the tubules. In these studies they furthermore found that when the animals were given an alkali by mouth the severity of the renal injury was diminished and that there occurred an increase in the output of urinary nitrogen.

Kingsbury and Bell (190) and Potter and Bell (191) continued these functional studies of the tartrate nephritis. They also observed an early suppression of phenolsulphonephthalein elimination and no definite interference with hippuric acid synthesis. With such functional findings there existed a severe necrosis of the convoluted tubule cells with no injury to the glomeruli and collecting tubules. In other of their experiments in which there existed the same type of injury they found the kidney unable to eliminate not only phenolsulphonephthalein but indigo carmine and methylene blue. The excretion of lactose was twice as long, and from potassium iodide four times as long as in the normal kidney. They conclude that the above mentioned dyes are excreted by the convoluted tubules and that lactose and potassium iodide are excreted by the glomeruli.

In 1917 Karsner (192) studied the vascular reaction of the kidney in an acute tartrate nephritis employing the technique previousiy outlined by Schlayer and his co-workers. He found that on the second or third day of such a tubular injury the vascular reaction of the kidney was normal to constrictor and dilator stimuli but that caffein with adequate vasodilatation failed to induce urine formation as in the normal dog. By the fifth day of the nephritis the vascular response was more marked and associated with this change there was an increased effect from caffein.

Baer and Blum (193) in 1907 first made the observation that glutonic, tartaric acids and their salts greatly inhibited the elimination of urinary nitrogen and dextrose in dogs with a phlorhizin diabetes. They offered no explanation for this action. In 1912 Underhill (194) in studying the influence of sodium tartrate on the elimination of various urinary constituents during phlorhizin diabetes ascribed their retention to the same tubular nephritis which the tartrates induce. Much later than this Pearce and Ringer (195) in similar metabolic studies noted the reduction of urine and the anuria first described

by Underhill. They extended their observations to normal dogs, with a study of the toxic effect of the tartrates when administered by mouth, intraperitoneally and subcutaneously. These experiments confirmed the work of previous students of this poison, that it selects the convoluted tubule epithelium for its dominant action. In about half of the animals they observed an exudate into the subcapsular spaces of the glomeruli.

The more recent work of Underhill and Greenhouse (196) and of Underhill and Ferguson (197) has been concerned with the blood concentration in tartrate nephritis and of the ability of such an injury to render animals susceptible to potassium chloride. The blood underwent a dilution during the course of the tartrate injury which these observers consider may explain the anemia. In their studies with potassium chloride they found that the renal injury did not render the animals more susceptible, which is contrary to the findings of Smille in his work with potassium toxicity in animals with a uranium nephritis. Umeda and Ringer (198) have suggested that both tartaric and oxalic acids exert their action on the renal tubules through the precipitation of calcium salts in these cells during the course of their elimination. Underhill however has shown that only a small amount of the salts of tartaric acid leaves the body through the kidney which tends to minimize the suggestion of Umeda and Ringer concerning the way in which this toxic action is induced.

Unlike the study of most nephrotoxic agents, there appears to be very nearly a unanimity of opinion that the salts of tartaric acid exert a definite selective affinity for the convoluted tubule cells. When such an isolated injury has developed in the kidney, there may be for a period a continued excretion of water and sodium chloride, but the output of all of the dyes employed as renal functional tests is arrested, and there occurs a nitrogen retention. At such a period of renal functional response the glomeruli appear normal and according to Karsner's investigations have a normal vasodilator and vasoconstrictor response. Such observations certainly strengthen the theory of active secretion on the part of the specialized cells found in the convoluted tubules.

VII. THE ACUTE NEPHRITIS FROM BACTERIA, BACTERIAL TOXINS, SERA AND FOREIGN PROTEINS. With the recognition in the early eighties of the importance of bacteria as the cause of disease, it was only natural that these agents should be looked upon as the likely cause of various types of nephritis. Excepting a few of the acute

and chronic glomerulonephrites, clinical observation and experimental methods have only been able to establish a specific connection between bacteria and a few renal injuries diffuse enough in character to be classed as a true nephritis.

As early as 1884, Ernst (199) suggested that a true nephritis was an inflammatory state likely caused by infectious agents. In 1894 Pernice and Scagliosi (200) inoculated rabbits, dogs and guinea pigs subcutaneously and intravenously with a variety of organisms, as anthrax, pyocyaneus, prodigiosus and the Staphylococcus aureus. They claimed to have induced a glomerulonephritis and considered the toxins of the bacteria to have much less effect on the kidney than the bacteria.

Morse (201) injected rabbits with dead organisms and toxin from a highly virulent staphylococcus obtained from cases of osteomyelitis. The animals showed a renal epithelial injury and several a connective tissue hyperplasia. Lubarsch (202) at about the same time succeeded in inducing amyloid changes in the kidney vessels of animals by using living cultures of the staphylococcus, while Mannerberg (203) isolated the same organism from the urine of patients with acute nephritis and made intravenous injections of the cultures into rabbits and dogs with the production of an acute nephritis. The organisms failed to proliferate in the kidney so that the initial injury became chronic with the disappearance of the bacteria from the urine.

Aschs (204) in order to be certain that toxin or dead bacteria reached the kidney in sufficient concentration and number, injected the toxin or infectious agents into the renal artery, a method more recently employed by Winternitz (205). Aschs succeeded in producing a renal injury of an indiscriminate type, while the experiments of Winternitz resulted in a general infection. The infection subsided while the kidney changes progressed and induced a severe renal impairment characterized by acute, subacute, and chronic changes which could be classed as types of a glomerulonephritis.

In 1890 Babes (206) described in an incomplete fashion the degenerative changes induced in the kidneys and livers of rabbits from diphtheria toxin which was followed in 1891 and 1892 by the work of Welch and Flexner (207), (208) and later by the study of Lyon. These papers will be considered in a discussion of the nephritis from diphtheria toxin.

LeCount and Jackson (209) and Hartzell and Henrici (210) effected renal injuries by the use of staphylococci which were fairly diffuse

in character and yet of such a nature that it is questionable whether or not they should be considered as true nephritic processes. The experiments of Major (211) appear more convincing. He injected rabbits with a strain of bacillus mucosus capsulatus. One injection resulted in the production of a diffuse hemorrhagic type of nephritis with necrosis of the tubular epithelium, while repeated smaller injections induced a chronic productive type of nephritis.

The experimental production of an acute renal injury by the use of bacteria which is diffuse enough in character, and comparable in its pathology to the type of nephritic process which develops in human material from bacterial invasion is certainly extremely difficult to obtain. Some acute, and often a severe renal injury occurs from such experimental procedures, and in the healing process a chronic and not infrequently a fairly diffuse injury arises comparable in a measure to some of the chronic types of nephritis observed in man, but the acute renal changes produced by the introduction of living or dead bacteria, have with few exceptions been unsatisfactory.

The production of an acute nephritis by diphtheria toxin has been obtained by numerous investigators. Following the early work of Babes, Welch and Flexner, Flexner (212) again studied the action of this toxin and found it to induce a necrosis of the glomerular capillaries with a leucocytic infiltration. Lyon (213) made a detailed study of the nephrotoxic action of the toxin when it was diluted with salt solution and injected in lethal and sublethal doses into the ear veins of rabbits. Many of the animals recovered from such injections and in them he could not demonstrate a chronic renal injury.

In the acute and subacute intoxications the renal lesions were the same, but varied in intensity. The urine contained albumin, casts and later blood. The kidneys in the acute nephritis from the toxin were enlarged and hemorrhagic with a mottled surface. The cortex was increased in width and of a dark red color. Microscopically the glomeruli showed an intense congestion with occasional hemorrhage. The glomerular lesions depended upon the duration of the injury. The early changes were characterized by an intense dilatation of the loops, many of which were occluded by thrombi. Hyaline thrombi were found in the glomerular arteries. The endothelium of the capillaries was swollen, and between the tufts and in the subcapsular space there was a lecucocytic infiltration. The tubular epithelium was granular and swollen to the point of occluding the lumen of the

tubules. The convoluted tubule cells were not so uniformly involved as those of the ascending limb of Henle's loops. These cells became necrotic.

Frothingham (214) also produced glomerular and arterial lesion in rabbits from the use of diphtheria toxin and largely confirmed the work of Lyon. Like Lyon, he was unable to obtain permanent chronic lesions.

Tribe and Barcroft (215) induced both a vascular and epithelial injury in animals from the use of this toxin and found the flow of blood through the kidney reduced and oxygen consumption diminished. Karsner and Dennis (116) obtained the same type of injury and observed a nitrogen retention, while Eisenbrey (217) demonstrated in this type of nephritis a marked reduction in the elimination of phenolsulphonephthalein.

In 1917 Smith working with Ophüls, (218) Faber (219), (220) and Faber and Murray (221), employed various bacteria as well as diphtheria toxin to produce an acute nephritis. Smith and Ophüls inoculated rabbits intravenously with streptococci and obtained a definite glomerulo-nephritis which also involved the tubules. Here the cells were granular, vacuolated and fatty.

Faber and Murray employed not only streptococci but the colon bacillus, streptococcus salivarius and the staphylococcus aureus. With these organisms they induced a fairly pure type of glomerulo-nephritis even when immune bodies could be demonstrated in the blood serum. Faber induced a nephritis in young rabbits with diphtheria toxin which at first was purely glomerular. The later changes in the tubular epithelium he considers to be secondary to the extensive glomerular damage. In Faber's second paper he used a combination of the toxin with Bacillus coli and obtained a marked difference in the type of nephritis. The glomerular injury was more extensive and of a hemorrhagic character. This additional characteristic of the injury he attributes to bacteriolysis of the colon bacillus in the capillary tufts with the liberation of toxic groups contained in its protein molecule.

The toxic effect of foreign proteins and various sera for the kidney has been recognized for some years. Only recently through the important experiments of Longcope has it attracted the attention it deserves.

In 1898 Sollmann and McComb (222) studied the effect of injecting extracts of rabbit muscle into rabbits, and obtained an

albuminuria with severe epithelial degeneration. Sollmann and Brown (223) studied the effect of injections of egg albumin and other proteins in rabbits and dogs. In many of the animals the use of egg albumin induced a free diuretic effect while in others there was a hemoglobinuria followed by an anuria. In the dogs there was no epithelial degeneration, while in the rabbits the cells of the convoluted tubules were cloudy, edematous and vacuolated.

Later than these experiments Pfaff and Vejux-Tyrode (224) obtained an albuminuria when animals were injected with their own blood. Pearce (225) prepared extracts of normal rabbits' kidneys several days after they had been injected with egg albumin and horse serum, and found the extracts to have the ability to sensitize guinea pigs to second injections of these proteins. From these observations he concluded that foreign proteins tend to accumulate in the kidney. Such a reaction he found to be delayed if the animals were rendered acutely nephritic by uranium or potassium dichromate.

As early as 1901, Bierry (226) had shown that the serum of nephritic animals when inroduced into a normal animal would effect a renal injury. Later than this Pearce and Sawyer (227) rendered rabbits and dogs acutely nephritic with either potassium dichromate or uranium and found the serum from the dogs to have a toxic action on the kidneys of normal dogs which was shown by the appearance of albumin and casts in the urine. No nephritic action was obtained when the serum of rabbits was used. This work was followed by that of Pearce and Eisenbrey (228) in which they studied the vascular response of the kidney following the use of nephrotoxic and hemolytic immune sera. Such sera were found to induce an acute exudative type of glomerular injury which was not indicated by physiological methods. The glomeruli responded in a normal manner to constrictor and dilator stimuli.

The investigations of Longcope (229), (230) show the specific character of certain foreign proteins in inducing a nephritis and are suggestive of many similar reactions which may arise through the action of toxic molecules from the proteins of bacterial and other cells. Longcope used a variety of animals and sensitized them with horse serum or egg albumin. Second injections in such animals induced an acute nephritis characterized by degeneration and necrosis of the epithelium of the loops of Henle, the collection tubules, and more rarely the convoluted tubules.

The repair changes from such injuries consisted in the formation of connective tissue and chronic changes in the glomeruli. Wilson and Oliver (231) have obtained very similar results through the intravenous injection of animals of 1 to 2 cc. per kilogram of inactivated anti-kidney serum. The cells of the loops of Henle were most involved in the nephritis.

Stoddard and Woods (232) have obtained even more specific renal reactions by the use of various bacterial poisons such as Vaughn's split products of the tubercle bacillus which they found to cause necrosis of the convoluted tubules, while the injection into rabbits of an extract obtained from powdered staphylococci and streptococci induced a degeneration with marked vacuolation of the epithelium in the middle portion of the same tubules.

It is likely too early, and experimental results are insufficient, to size up the importance of foreign proteins as nephrotoxic agents. The fact however has been demonstrated that such substances will induce an acute renal injury, and furthermore, that different proteins induce different types of such an injury. Such findings may turn our attention away from specific bacterial toxins as nephrotoxic agents, and increase our interest in the toxic properties of the protein molecule of the bacterial cell and the protein found in food material.

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RECENT ADVANCES IN BIOLUMINESCENCE

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This review will give a brief account of the physical, chemical and physiological investigations of bioluminescence which have appeared since 1919 and a reference to some of the earlier work which was omitted from my book, *The Nature of Animal Light* (J. B. Lippincott, Philadelphia, 1920).

The literature list includes many papers not referred to in the text. Together with the bibliography in my book, and that in Pratje's "Das Leuchten der Organismen," (Ergebnisse der Physiologie, vol. xxl), a fairly complete record of papers on bioluminescence may be obtained. The article of Pratje reviews all phases of animal luminescence in the various groups which produce light, and will interest the morphologist as well as the physiologist.

The light of living creatures is connected with oxidation processes and in several forms can be definitely proved to be due to the oxidation of luciferin to oxyluciferin in presence of an enzyme, luciferase. In other organisms the luciferin-luciferase reaction cannot be demonstrated, but we must believe that this is due to experimental difficulties, to unstable luciferin, or some secondary factor. It would seem, *a priori*, that luminescence in all forms, including bacteria, must be fundamentally the same, and an elucidation of the chemical mechanism involved may throw some light on the nature of animal oxidations in general, a subject now justly claiming the attention of workers in various branches of physiology. Since oxidation is the process, fundamental to both life and light, it will serve as a proper starting point for a discussion of bioluminescence.

LUMINESCENCE AND OXYGEN. Despite some recent observations to the contrary, all luminous animals require dissolved gaseous oxygen for luminescence. Kanda (1920 b) reported that Cypridina luminescence was not an oxidation but this statement was due to the use of a faulty method of investigation (Harvey, 1920). Very small amounts of oxygen are sufficient to give luminescence with Cypridina.

The minimum concentration of oxygen for luminescence by luminous bacteria has been measured by Harvey and Morrison (1923). By means of flow-meters, pure hydrogen and hydrogen containing a small percentage (0.34 per cent) of oxygen were mixed in definite proportions, which could be varied, and continuously bubbled through a suspension of the bacteria in sea water. The suspension was sufficiently dilute so that the bacteria used up a negligible amount of the oxygen passing through the solution and in equilibrium with the solution. It appears that one part oxygen in about 143,000 parts hydrogen by volume, or 0.0053 mm. Hg oxygen pressure is sufficient to give a visible luminescence of bacteria. This corresponds to 1 part oxygen by weight dissolved in 3,700,000,000 cc. sea water. Part of this oxygen is no doubt used for respiratory oxidations, so that only a certain (unknown) percentage is actually utilized in luminescence. A mixture of Cypridina luciferin and luciferase will give a visible luminescence in still smaller concentrations of oxygen than the bacteria. No figure can be given as the amount is so low as to necessitate a rearrangement of the apparatus used for luminous bacteria, in order to obtain a measurement.

Kanda (1920 c) has made experiments to prove that the luminescence of the fire-fly is an oxidation, but this fact certainly requires no further proof than given by many previous researches. It was observed that the excised fire-fly light organs, when evacuated, gave no light, but that when nitrogen containing 1 per cent oxygen was admitted, a brighter light appeared than in light organs in air containing 20 per cent oxygen, *but not previously evacuated*. This is explained as an effect of stimulation of the organ by the readmitted gas or a change in the surface of contact of the oxidizable material for the readmitted gas. I think the effect is possibly due to the increased pressure forcing open the tracheoles at the tracheal end cells, thus bringing the gas directly to the photogenic cells (see p. 655).

The oxygen used by the light organs of 60 female Luciola vitticollis, separated as much as possible from other tissue, was found to be 6.01 cc. in 25 hours. The CO_2 developed was 5.66 cc. (Kanda, p. 147). One would like to know the weight of the tissue in order to compare those results with the respiratory rates of other isolated insect tissues. It is my belief that a very small amount of oxygen is actually used for luminescence but this important point still awaits experimental determination.

CHEMICAL NATURE OF LUCIFERIN AND LUCIFERASE. In order to understand the significance of "luciferin and luciferase solutions," discovered by Dubois in 1887, it is necessary to recall that luciferin solution is prepared by making a hot water extract of a luminous organ. Heating destroys the luciferase but does not harm the luciferin. Luciferase solution is prepared by making a cold water extract of a luminous organ, when both luciferin and luciferase dissolve and luminescence occurs. The extract is then allowed to stand in the air till the light disappears, evidence that the luciferin has been completely oxidized to oxyluciferin leaving the luciferase, an enzyme, in solution. A luciferase solution, by virtue of this mode of preparation, must contain oxy-luciferin as well as luciferase. Some of the properties of luciferin and luciferase are well known.

Luciferase. Cypridina luciferase is non-dialyzable; destroyed by trypsin; insoluble in alcohols and all fat solvents; soluble in water, dilute salt, acid and alkali solutions; practically completely precipitated by phosphotungstic, tannic and picric acid and basic lead acetate; salted out by saturation with $(NH_4)_2SO_4$ but not by NaCl, and readily adsorbed on fine particles.

Kanda's work (1921) on luciferase has confirmed many of my earlier results but not all of them. Out of eleven reactions on luciferase (Kanda, table, p. 7, 1921) carried out by both of us, where Kanda feels sure of the result, two disagree. One of these (salting out by $MgSO_4$) is so near an agreement that differences in temperature, acidity of solution, or dark adaptation of the eye may explain the result. I find luciferase "nearly completely precipitated" while Kanda finds it "completely precipitated" on saturation with $MgSO_4$.

The point in which our results disagree most is the effect of half-saturation of luciferase with $(NH_4)_2SO_4$. I find it slightly precipitated while Kanda finds it "completely precipitated." In discussing these "salting out" experiments it should be pointed out that Kanda used an "experimental solution" prepared by precipitating a water extract of Cypridina with $HgCl_2$ until a drop of $HgCl_2$ solution added to the filtrate gave no further precipitate. Luciferase is not precipitated by $HgCl_2$ (not completely, at least), and is found in the filtrate together with $HgCl_2$. In my experiments the water extract of Cypridina itself was used for salting out, without previous treatment with $HgCl_2$.

Repeating my original experiments, on salting out aqueous luciferase extract by half-saturation with $(NH_4)_2SO_4$, I again find luciferase *only partially precipitated.* On salting out a $HgCl_2$ solution

of luciferase by half-saturation with $(NH_4)_2SO_4$ I find nearly complete precipitation, practically the result which Kanda has observed.

The $HgCl_2$ seems to effect the activity of the luciferase and this may explain why salting out is more complete in its presence. On the other hand, Kanda may be salting out the mercury salt of luciferase while I am salting out luciferase itself. Whatever the explanation, I hold that the use of an untreated extract of Cypridina gives more significant information regarding the properties of luciferase than an extract first precipitated by $HgCl_2$ and containing excess of Hg.

Kanda is inclined to consider luciferase a globulin perhaps combined with a carbohydrate gum. I have dialyzed luciferase for many days against many changes of distilled water and find that it is still soluble. Insolubility in water but solubility in salt solution defines a globulin. All workers in protein chemistry will agree that the classification of proteins by solubility is a make-shift classification and that salting out distinctions are arbitrary. These are the only means of classification we have at present, and upon this classification, if luciferase is a protein, we must place it among the albumins. Protein color tests on "luciferase solutions" have, in my opinion, no significance for deciding the protein nature of luciferase.

It would be of great interest to know if antibodies could be formed for luciferase. Kanda injected crude solutions of luciferase into the ears of rabbits and found that the serum of the animals gave a slight precipitate with luciferase solutions. There was so small a precipitate, and as the solution injected contained natural proteins of the animal, the significance of the results is uncertain. Injecting luciferin solutions gave inconclusive results likewise.

Luciferin. Cypridina luciferin is slowly dialyzable; not destroyed by trypsin; soluble in absolute methyl, ethyl, propyl alcohol and acetone but insoluble in most fat solvents; soluble in water, dilute salt, acid and alkali solutions; practically completely precipitated by phosphotungstic but not by picric acid; practically completely salted out by $(NH_4)_2SO_4$ but not by $MgSO_4$ or NaCl, and readily adsorbed on fine particles.

The recent work on luciferin is also by Kanda (1924) and a distinct advance is made in the isolation and identification of this substance. By extracting dried fat-free *Cypridinae* (after ether and petroleum-ether treatment) with methyl alcohol, evaporating to a small volume and adding ethyl alcohol, a precipitate is produced which gives protein color reactions but contains no luciferin. This is filtered off and

the filtrate (containing luciferin) evaporated almost to dryness and taken up in water saturated with H_2 to prevent spontaneous oxidation of the luciferin. Saturation with $(NH_4)_2SO_4$ completely precipitates the luciferin and this precipitate is then redissolved in water saturated with H_2. Such a solution gives negative biuret, tryptophane, xanthoproteic, Millon, and ninhydrin reactions, although it contains luciferin. This is certainly good evidence that luciferin is not a protein, provided there is a sufficient concentration of luciferin present to respond to protein color reactions. It is impossible to definitely decide this question, but it must be borne in mind that the luciferin in one part (by weight) of *whole dried Cypridinas* in one million parts of sea water will give a bright light with luciferase. Protein color tests are not as sensitive as this.

Kanda states that purified luciferin solution is positive to the Molish test for carbohydrates. This statement is also made in the conclusions, although "redistilled water which the writer used gave always the Molish reaction without the luciferine even though it was always distilled thrice." (Kanda 1924, p. 442). One wonders why any reference to the Molish test is made since distilled water also gave the Molish test.

The purified solution of luciferin prepared by Kanda's method is completely precipitated only by saturation with $(NH_4)_2SO_4$, apparently not precipitated by the alkaloidal reagents, although the interpretation becomes somewhat difficult as the luciferin is neither found in the precipitate which appears on adding phosphotungstic acid nor in the filtrate. Phosphotungstic acid either harms the luciferin, or, I believe more probable, spontaneous oxidation of the luciferin without luminescence has occurred.

I have often been discouraged in attempting to interpret the results of work with luciferin; first, because of the small concentration of luciferin detectable by light production and, second, because of the rapid spontaneous oxidation of the material. Luciferin might be precipitated by a substance and yet the precipitate, when tested with luciferase, show no luciferin present, because it had spontaneously oxidized to oxyluciferin.

Whether luciferin is a protein or not, cannot be decided at the present time. Kanda is conservative in coming to no conclusion, but certainly the evidence against its protein nature is accumulating. I refer the reader to original communications for further properties of luciferin, and to draw his own conclusions.

Reduction of oxyluciferin. The oxidation product of luciferin seems to represent a relatively slight change in the luciferin molecule, since it is readily reduced to luciferin again. These reduction methods involve the taking up of hydrogen and we may suppose that some such equation as the following expresses the change occurring:

$$LH_2 \text{ (luciferin)} \leftrightharpoons L \text{ (oxyluciferin)} + H_2 - X \text{ Cal.}$$

$$H_2 + O \rightleftharpoons H_2O + 58 \text{ Cal.}$$

Dehydrogenations are endothermic but the subsequent oxidation of the hydrogen no doubt frees enough heat to make the oxidation of luciferin exothermic. The temperature change per gram of luciferin oxidized or per gram of oxygen used up is an important determination, but the experiments conducted thus far only enable us to say that it is very small.

Some interesting effects can be obtained by reduction of a mixture of oxyluciferin and luciferase in salt solution by the electric current. Thus a metal cathode placed in such a solution will luminesce during the passage of a current. The anode is perfectly dark. The luminescence appears when the potential across the electrodes is in the neighborhood of 2 volts and is due to the film of active hydrogen that appears over the cathode. This reduces the oxyluciferin to luciferin, which then luminesces in presence of luciferase and oxygen, in the outer layer about the electrode.

Those metals (Mg, Al, Mn, Zn, Cd) which tend to liberate hydrogen from water, luminesce spontaneously in an oxyluciferin-luciferase-solution. The film of active hydrogen over their surface reduces the oxyluciferin as before. By touching these metals with a metal higher in the electrochemical series (as Fe, Cu, Ag, Pt), we form a galvanic cell and the Fe, Cu, Ag, or Pt now luminesce while the Mg, Al, Mn, Zn or Cd cease to luminesce. The more "noble" metal now forms the cathode of a galvanic cell at which reduction processes occur.

Oxyluciferin is also reduced at the surface of hydrogen catalysts. Platinized or palladinized surfaces in contact with hydrogen will reduce oxyluciferin readily. Such surfaces glow when placed in oxyluciferin-luciferase solution provided they have first been saturated with hydrogen. No glow appears if they are first exposed to oxygen. As in the experiment with the electric current, a layer of oxyluciferin next to the Pt surface is reduced and then reoxidized with light production in the outer layer containing oxygen and luciferase. The principle is important as it indicates how a continuous luminescence

might be produced by simultaneous reduction of oxyluciferin and oxidation of luciferin in two regions of a lamp.

In a previous paper (Journ. Gen. Physiol., ii, 1920, 207) I stated that oxyluciferin solution in quartz tubes exposed to ultraviolet light would be reduced with formation of some luciferin. There are so many chemical reactions that proceed in the presence of ultraviolet light which might result in formation of reducing bodies that I doubt if we can conclude that light itself favors the reduction of oxyluciferin.

The various views which have been suggested to represent the mechanism of luminescence are as follows:

Dubois, (Compt. rend. Soc. Biol., 1918, lxxxi, 317.)

Co-luciférase + preluciférine = luciférine

Luciférase + luciférine = oxyluciférine

Oxyluciférine + oxygéne = lumiérè

Gerretsen, (1922)

$$\text{Nahrstoff} \rightarrow \underset{\text{Photogenase}}{\text{Leuchtstoff}} \rightleftharpoons \underset{\text{Luciferase}}{\text{Oxyleuchtstoff}} + \text{Licht}$$

Harvey

Preluciferin→ luciferin.

Luciferin + luciferase⇄luciferaseluciferin.

Luciferaseluciferin⇄oxyluciferin+H_2 + luciferase (with luminescence)

$H_2 + 0 = H_2O$

In the absence of luciferase, luciferin will oxidize to oxyluciferin but no light is produced. This is the basis for an assumption of a combination between luciferase and luciferin preparatory to luminescence. As I have already pointed out the oxidation—reduction system is similar to that of dyes, such as leuco-methylene blue—methylene blue.

SPECIFICITY OF LUCIFERIN AND LUCIFERASE. The luciferin-luciferase reaction in Cypridina is obtained with very minute amounts of these substances in solution. By dilution experiments it is possible to show that if dried Cypridina contains 1 per cent of luciferin, then one part luciferin in 40 billion (4×10^9) parts sea water will give a just visible light when mixed with luciferase. Making the same assumption of 1 per cent luciferase in the dried animal, one part luciferase in 8 billion parts of sea water will give a visible luminescence when mixed with luciferin (Harvey, 1923).

In the fire-fly, concentrated solutions, and in Pholas, a mollusc, fairly concentrated solutions of luciferin and luciferase are necessary to give a visible light when mixed. In some luminous animals no luciferin or luciferase can be demonstrated. One is not surprised at

TABLE 1

ORGANISM	"CROSS"					REACTION
Bacteria	Cypridina	luciferase	+	Bacteria	luciferin	Negative
	Cypridina	luciferin	+	Bacteria	luciferase	Negative
	Bacteria	luciferin	+	Bacteria	luciferase	Negative*
Cystoflagellates	Cypridina	luciferase	+	Noctiluca	luciferin	Negative
	Cypridina	luciferin	+	Noctiluca	luciferase	Negative
	Noctiluca	luciferin	+	Noctiluca	luciferase	Negative
Medusae	Cypridina	luciferase	+	Aequorea	luciferin	Negative
	Cypridina	luciferin	+	Aequorea	luciferase	Negative
	Aequorea	luciferin	+	Aequorea	luciferase	Negative
	Cypridina	luciferase	+	Mitrocoma	luciferin	Negative
	Cypridina	luciferin	+	Mitrocoma	luciferase	Negative
	Mitrocoma	luciferin	+	Mitrocoma	luciferase	Negative
Pennatulids	Cypridina	luciferase	+	Pennatula	luciferin	Negative
	Cypridina	luciferin	+	Pennatula	luciferase	Negative
	Pennatula	luciferin	+	Pennatula	luciferase	Negative
	Cypridina	luciferase	+	Cavernularia	luciferin	Negative
	Cypridina	luciferin	+	Cavernularia	luciferase	Negative
	Cavernularia	luciferin	+	Cavernularia	luciferase	Negative
	Cypridina	luciferase	+	Ptylosarcus	luciferin	Negative
	Cypridina	luciferin	+	Ptylosarcus	luciferase	Negative
	Ptylosarcus	luciferin	+	Ptylosarcus	luciferase	Negative
Ctenophores	Cypridina	luciferase	+	Bolina	luciferin	Negative
	Cypridina	luciferin	+	Bolina	luciferase	Negative
	Bolina	luciferin	+	Bolina	luciferase	Negative

Annelids	Cypridina	luciferase	+	Tomopteris	luciferin	Negative
	Cypridina	luciferin	+	Tomopteris	luciferase	Negative
	Tomopteris	luciferin	+	Tomopteris	luciferase	Negative
	Cypridina	luciferase	+	Odontosyllis	luciferin	Negative†
	Cypridina	luciferin	+	Odontosyllis	luciferase	Negative†
	Odontosyllis	luciferin	+	Odontosyllis	luciferase	Positive
	Cypridina	luciferase	+	Polynöe	luciferin	Negative
	Cypridina	luciferin	+	Polynöe	luciferase	Negative
	Polynöe	luciferin	+	Polynöe	luciferase	Negative
	Cypridina	luciferase	+	Chaetopterus	luciferin	Negative
	Cypridina	luciferin	+	Chaetopterus	luciferase	Negative
	Chaetopterus	luciferin	+	Chaetopterus	luciferase	Negative
	Cypridina	luciferase	+	Polycirrus	luciferin	Negative
	Cypridina	luciferin	+	Polycirrus	luciferase	Negative
	Polycirrus	luciferin	+	Polycirrus	luciferase	Negative
Crustacea	Cypridina	luciferase	+	Pyrocypris	luciferin	Positive
	Cypridina	luciferin	+	Pyrocypris	luciferase	Positive
	Pyrocypris	luciferin	+	Pyrocypris	luciferase	Positive
	Cypridina	luciferase	+	Meganyctiphanes	luciferin	Negative
	Cypridina	luciferin	+	Meganyctiphanes	luciferase	Negative
	Meganyctiphanes	luciferin	+	Meganyctiphanes	luciferase	Negative†
	Cypridina	luciferin	+	Cypripina (?)	luciferin	Positive
	Cypridina	luciferin	+	Cypripina (?)	luciferase	Positive
	Cypripina	luciferin	+	Cypripina (?)	luciferase	Positive
Myriapods	Cypridina	luciferase	+	Geophilus	luciferin	Not tried
	Cypridina	luciferin	+	Geophilus	luciferase	Not tried
	Geophilus	luciferin	+	Geophilus	luciferase	Negative‡

*Gerretsen (1922–23) has reported positive results with Photobacterium javanense luciferin and luciferase but negative with Photobacterium phosphorescens.

†Perhaps a slight reaction.

‡Rather dilute solutions.

TABLE 1—*Concluded*

ORGANISM	"CROSS"					REACTION
Insects	Cypridina	luciferase	+	Luciola	luciferin	Negative
	Cypridina	luciferin	+	Luciola	luciferase	Negative
	Luciola	luciferin	+	Luciola	luciferase	Positive
Molluscs	Cypridina	luciferase	+	Pholas	luciferin	Negative
	Cypridina	luciferin	+	Pholas	luciferase	Negative(?)
	Pholas	luciferin	+	Pholas	luciferase	Positive
Balanoglossid	Cypridina	luciferase	+	Ptychodera	luciferin	Negative
	Cypridina	luciferin	+	Ptychodera	luciferin	Negative
	Ptychodera	luciferin	+	Ptychodera	luciferase	Negative
Ascidians	Cypridina	luciferase	+	Pyrosoma	luciferin	Negative
	Cypridina	luciferin	+	Pyrosoma	luciferase	Negative
	Pyrosoma	luciferin	+	Pyrosoma	luciferase	Negative
Fish	Cypridina	luciferase	+	Photoblepharon	luciferin	Negative
	Cypridina	luciferin	+	Photoblepharon	luciferase	Negative
	Photoblepharon	luciferin	+	Photoblepharon	luciferase	Negative
	Cypridina	luciferase	+	Anomalops	luciferin	Negative
	Cypridina	luciferin	+	Anomalops	luciferase	Negative
	Anomalops	luciferin	+	Anomalops	luciferase	Negative

that in luminous bacteria where the substances may be in a condition difficult of extraction ("endoluciferin" or "endoluciferase"), or in Chaetopterus, a worm, where the bulk of non-luminous material extracted is large compared to the luminous material, so that the concentration of luciferin or luciferase in the extract may be small, or the luminous substances may be unstable; but in many jelly-fish or pennatulids there is abundance of luminous secretion, which may be extracted and will give a bright luminescence upon dilution with sea water or addition of cytolytic agents, but never the luciferin-luciferase reaction.

Why luciferin and luciferase cannot be demonstrated in a jelly-fish is one of the outstanding problems in bioluminescence. It is possible that jelly-fish luciferin is destroyed by heat or that just enough luciferase is present in the jelly-fish to be completely used up by the luciferin (for even in Cypridina, luciferase is diminished on mixing with large quantities of luciferin). The latter could be tested by mixing luciferase of Cypridina with luciferin of the jelly-fish, provided the reaction is not specific, i.e., provided Cypridina luciferase may be assumed to react with jelly-fish luciferin. Unfortunately all the evidence points to a high specificity of the luciferin-luciferase reaction. Only if the luminous animals are very closely related is a mutual interaction of luciferin and luciferase with luminescence possible.

Table 1 gives the results of mixing luciferin[1] from one species with the luciferase from Cypridina and vice-versa.

Although Cypridina luciferin cannot be oxidized (with light production) with extracts of non-luminous animals or with oxidizing agents, Dubois finds that Pholas luciferin can be. He has recently (1923) described luminescence of Pholas luciferin on mixing with Fehling's solution and H_2O_2.

The behavior of extracts of luminous jelly-fish, pennatulids, and some other forms, which give no luciferin-luciferase reaction, is quite different from that of *Cypridina*. If one extracts a luminous jelly-fish with sea water and allows the extract to stand with frequent agitation until all the light diappears (i.e., if one treats the material as one would to prepare a luciferase solution), the sea water extract will give a bright luminescence upon addition of fresh water or various cytolytic agents, (Harvey, 1921 c). If one wipes off the luminous slime of a jelly-fish (Pelagia noctiluca) on filter paper and places the paper in pure

[1] By luciferin is meant a solution so prepared that it should contain luciferin. The same statement holds for luciferase.

$MgSO_4$ or KCl solution, isotonic with sea water, a bright luminescence also appears (Heymans and Moore, 1924). No luminescence appears in pure isotonic $MgCl_2$ or NaCl solution. These effects are obtained under conditions when no intact photogenic cells could be present, and Heymans and Moore do not believe they are connected with cytolysis. The effect of a series of salts was studied by these authors.

Under the microscope one can observe that the light is not homogeneous, but points or discs of light appear suddenly and then fade out (Harvey, 1921 c). It is as if granules were dissolving with emission of light. We may speak of granulolysis. Histological studies indicate that the photogenic substances occur in the form of granules in luminous cells. The light seems to accompany always the solution of these granules, and I have not been able to bring this phenomenon satisfactorily in line with the light production in Cypridina, where luciferin and luciferase solution can be separated and will react, even in very great dilutions, giving a homogeneous luminescence.

Concentrated extracts of Cypridina behave somewhat as the jellyfish. Cytolysis may occur in these concentrated extracts, and this probably explains my early statements that Cypridina luciferase would give light with non-oxidizable substances and with extracts of non-luminous animals. I believe Kanda (1920 a) is correct in holding that Cypridina luciferase is unable to oxidize substances in extracts of non-luminous animals with light production. All the data thus far accumulated point to a very great specificity of Cypridina luciferin and luciferase.

Dynamics of luminescence. It is of considerable interest to study the course of oxidation of luciferin, i.e., the reaction velocity curve. The logical method of attacking this problem is to start with a given concentration of luciferin and to determine the amount left after stated periods of time, as in the determination of any reaction velocity. Such a procedure is not possible with luciferin, as methods of analysis apart from the luminescence it gives are unknown. However, when luciferin and luciferase solutions are mixed, we get a bright luminescence which represents light due to some initial concentration of luciferin. After mixing, the luminescence gradually decreases in intensity and a study of this decay curve has given an interesting insight into the course of the oxidation of luciferin. Not only is photometry of relatively weak lights uncertain but becomes much more so if the light intensity is continually changing, continually falling off as it is during decay of Cypridina luminescence.

The problem is a difficult one but has been solved by Amberson (1922) by means of a photographic method. Amberson photographed the light on a moving picture film wrapped on the drum of an ordinary kymograph. The container for the luminescent mixture is a tube of glass, covered with black paint and adjusted very near the drum. On one side is a small slit, through which the light produced can shine directly on the film. A stirring device and thermometer are also placed in the tube and a pipette containing the luciferase is fixed in such a position that luciferase can be mixed at the proper time with luciferin, which is placed in the tube. With two such tubes it is possible to take two records at the same time on the same moving picture film. A calibration record must also be placed on the same film. If we take records in this way during revolution of the drum, we observe a streak of blackening on development, which gradually fades out as the film has revolved and the luminescence becomes less intense. Densities on the film are then read by an optical pyrometer, as light intensities. The calibration exposures are made in order to determine how much blackening a given intensity of light will produce on the film. They are obtained by allowing *Cypridina* luminescence to pass through neutral filters of known absorption before striking the film and must be impressed after the moving record is taken. The readings are consequently not absolute but relative, made in arbitrary units, and tell merely what intensity the light has reached after a given interval, as compared with that at the beginning. Oxygen is always present in such high concentration that it does not affect the light intensity even though its concentration may vary somewhat during the course of the reaction.

Curves obtained in this way are straight lines over a considerable temperature (5° to 35°C.) range, if we plot log intensity of luminescence against time. The light falls off in intensity with time in the same manner, as a hot body cools off. Since this decay curve is also the same as would be followed by a monomolecular reaction, when reaction velocity, dx/dt, is plotted against time, the conclusion is drawn that intensity of luminescence (within certain limits) is determined by reaction velocity and that only one molecule of luciferin is undergoing transformation. Plotting log concentration of A (luciferin) against time should also give a straight line for a monomolecular reaction but the experiments in which luciferase concentration or temperature is varied show that luminescence intensity is determined by reaction velocity, dx/dt, and not by concentration of luciferin at any given time.

The principal additional facts established by Amberson's work are:

1. Stirring does not affect the luminescence intensity or the form of the decay curve.

2. The decay curve (reaction velocity curve) falls off in proportion to luciferase concentration, i.e., the velocity constant is proportional to luciferase concentration.

3. Logarithmic plottings with two different concentrations of luciferin but with the same luciferase are parallel. The velocity constant is not affected but light intensity (reaction velocity) is naturally less with smaller concentrations of luciferin.

4. There is always an initial flash of light when the luciferin and luciferase are mixed, too high to agree with the remainder of the curve. Its meaning is unknown.

5. The temperature cofficient is high. In a subsequent paper[2] Amberson has collected the values obtained in nine experiments and finds Q_{10} for different 10° temperature intervals to average 2.74. There is a tendency for the higher (25° to 35°) intervals to have a greater Q_{10} than the lower (5° to 15°).

STIMULATION TO LUMINESCENCE. Only the luminous bacteria and fungi luminesce continually, day and night. All other luminous animals produce their light on stimulation, usually the mechanical agitation of the water in which they live. Some of the few fishes which possess a constant intensity light organ nourish symbiotic luminous bacteria in the organ (Harvey, 1922 c). Although these bacteria luminesce continually, the light from the organ can be turned on and off by a screening device. The movement of the screen (in Photoblepharon), or the rotation of the light organ itself (in Anamolops), is muscular, under nerve control. There is obviously no real problem of stimulation in these fishes. The real problem involved in stimulation to luminescence is why the oxidation of luciferin occurs only as a result of stimulation of the luminous cell itself, either directly or indirectly by nerves. In the above cases movement of muscles causes the light to appear or disappear.

In Cypridina the luminescence on stimulation is said to be due to the contraction of muscles which squeeze luciferin and luciferase out of separate gland cells. Meeting in the sea water, these bodies luminesce. The problem of stimulation is this case is no different from that in the above mentioned fishes. Again nerves stimulate muscles to contract. They do not act upon the luminous cells themselves.

[2] Not yet published.

In jelly-fish or some worms a luminous slime is secreted from gland cells. Stimulation of one part of the animal will cause a wave of luminescence to pass over it. There are luminous[3] nerves which excite gland cells to secrete. Luminous material is extruded to the exterior and luminesces in contact with oxygen of the sea water or perhaps luminous material begins to luminesce in the gland cell and is also extruded to the exterior. The problem in this case is largely the problem as to how stimulation brings about secretion.

In Noctiluca there are no nerves; the luminous material luminesces within the cell and *only upon stimulation*. We have here a true problem of stimulation of luminescence. There is no secretion of luminous material, no screening mechanism, no muscular squeezing of luminous substance in contact with oxygen. What does stimulation do to set off the luminscence? The problem is no doubt similar to the problem of any kind of stimulation. Why does a mechanical disturbance set off the mechanism by which a muscle contracts? We may imagine in the case of Noctiluca: (1) that stimulation admits oxygen to the cell by increasing cell permeability, (2) that stimulation removes some phase-boundary between luminous substances so that they come into contact with each other.

It can be shown (Harvey, 1922 b) that most cells are so readily permeable to oxygen that one cannot distinguish differences in permeability, even between dead and living cells. Permeability for oxygen would seem to be independent of stimulation. If the luminescence of a cell is dependent on increase in oxygen supply by stimulation, the mechanism for increasing oxygen must be an external secondary one and not an increase in permeability of the photogenic cell membrane itself. In the fire-fly, as we shall see, there appears to be a device for regulating the oxygen supply to the photogenic cells by opening the ends of the air tubes. Such a device is not present in the free swimming luminous protozoa like Noctiluca.

The most suggestive line of inquiry seems to point to some change in phase boundries in the cell bringing the substances concerned in light production in contact with each other. Noctiluca is undoubtedly one of the most favorable forms for investigation and much remains to be discovered in this field. It is quite obvious that stimulation of

[3] Luminous or photogenic nerves are of course not themselves luminescent, but their stimulation brings about luminescence of luminous cells, just as secretory nerves do not secrete, but their stimulation brings about secretion of gland cells.

luminescence must be individually investigated in each group of luminous animals and that structural conditions are most important in interpreting the result.

In a number of forms luminescence is rhythmic, flashing occurring at stated time intervals, often characteristic of the species. Flashing occurs in the fish Photoblepharon, where it is occasioned by rhythmic screening and unscreening of the light organ,but is best known in fire-flies and glow-worms. Even the egg of the fire-fly may show rhythmic periods of luminescence and darkness, just before the larva hatches. Rhythmic flashing is under nerve control and a nerve cell rhythm, not a luminous cell rhythm. This is shown by cutting the organ away from the ventral nerve cord, when rhythmic flashing never occurs. However, the organ will flash when stimulated.

Sometimes all the fire-flies on one tree will flash rhythmically and synchronously. I have never seen this phenomenon myself but it undoubtedly occurs in some tropical fire-flies. There are too many positive references in the literature to doubt the truth of the observation (Gudger, 1919).

Snyder (1920) has observed that the rhythmic flashing in Photinus pyralis changes with temperature according to the Vant Hoff chemical coefficient. Between 19° and 29° the rate of flashing changes from 8 to 16 per minute. At a given temperature all insects flash at about the same rate with an extreme deviation of perhaps 10 per cent. Once started flashing in unison they would remain in unison at a given temperature, but the constancy of the rhythm at one temperature does not explain its synchronism. We must look to some peculiar behavioristic reaction of the tropical fire-fly itself to explain the flashing in unison.

Although we say that luminous bacteria produce a continuous light, no one really knows if this is the case or not. The effect of a continuous light would be given by innumerable minute points, each alone invisible, flashing rhythmically but not synchronously. Since we cannot see the light from a single bacterium, it may very well be that a single bacterium flashes rhythmically. The possibility does not seem likely, however, in view of the utter independence of the light of bacteria and stimulation.

The mechanism of flashing in the fire-fly is intimately connected with the structure of the organ. A mass of luminous cells on the ventral surface is backed by a "reflector" layer of cells containing minute crystals of some purin body, acting as a matt surface. It is

stated that the crystals increase during the life of the fire-fly and represent an oxidative decomposition product of the luminous material but I think this statement is far from proved. The photogenic layer receives a rich tracheal supply, the air tubes passing through tracheal end cells and ending in the luminous cells themselves. Nerves enter the photogenic layer and the consensus of opinion is that they are distributed to the tracheal end cells. On viewing the organ microscopically at night, one can see that the brightest light comes from the cytoplasm of the photogenic cell layer next to the tracheal end cells. Luminescence is undoubtedly intracellular in the fire-fly.

Lund (1911) showed that the flashing was not connected with the respiratory rhythm or any movement of dorso-ventral muscles. An excised organ does not flash spontaneously because the ventral nerve cord, which carries the stimuli, has been cut, but an excised organ is highly irritable and responds to local stimulation by a local flash of light.

Sudden increased air pressure applied to a dark excised organ will cause a flash (Lund, 1911). It looks as if the increased pressure had forced a valve, previously closed, and brought air in contact with the photogenic cells. Dahlgren (1917), has described such a complex valve mechanism in the tracheal end cell.

Gerretsen (1922) comes to the conclusion that periodic lighting is due to the admission of more oxygen to the organ by expansion of the tracheal end cells under nerve stimulation. Electrical stimulation of the ventral nerve cord, where thorax joins abdomen, results in a flash of light. This cannot be due to the manufacture of more photogen under nerve control since this implies very little photogen originally, whereas there is much photogen in the organ. Then nerve stimulation must affect the admission of oxygen. In chloroform narcosis of a fire-fly, we have first reversible quenching of power of flashing and, later, uniform luminescence of the organ. The first stage corresponds to the effect of chloroform in bringing about tonic contraction of tracheal end cells with consequent shutting off of oxygen from the cells, while the later uniform luminescence is due to secondary relaxation of the end cells (with continuous admission of oxygen), which has its counterpart in the effect of chloroform on muscles.

If this explanation of flashing is correct, it would seem possible to observe movement in tracheal end cells under the microscope and obtain conclusive proof of the hypothesis.

EFFECT OF LIGHT ON LUMINESCENCE. A survey of living forms discloses some luminous animals which emit light on stimulation quite independently of previous illumination (Chaetopterus, Ptylosarcus), some whose luminscence is inhibited by light but which will luminesce after a time in the dark (Ctenophores, Renilla, Noctiluca), and some whose luminescence can only be obtained at night whether they have been previously kept in the dark or not (Pelagia, Ptychodera). The latter show a true nocturnal and diurnal rhythm.

As the stimulation of most luminous forms involves a nerve mechanism, the question at once arises whether light acts on nerves, inhibiting stimulation, or upon the photogenic cells, inhibiting stimulation, and if the latter, whether the stimulation process is interfered with or the accumulation of the photogenic substance itself. Can the light also cause the disappearance of photogenic material?

The coelenterates are favorable forms for experiment. The propagation of a wave of luminescence over a colony of Pennatula or Cavernularia is a well known and a beautiful spectacle. Parker (1920) has observed in Renilla a rate of about 7.5 cm. per second at 20°, which agrees closely with the rate of a wave in Renilla causing withdrawal of zoöids and is no doubt a nerve impulse rate. The wave can pass over non-luminous areas and excite zoöids to luminescence some distance away.

Parker (1920) finds the luminous material in Renilla to be a white chalky substance forming masses at the base of the zoöids. It is associated with a yellowish crystalline material, but in favorable locations can be separated from the yellowish substance which is non-luminescent. When brought into a dark-room from daylight, Renilla does not luminesce until about one-half hour after it has been in the dark. At night the light is of course bright, but exposure to a 40 watt mazda lamp at 40 cm. caused the Renilla luminescence to become less intense but never to disappear completely. One would like to know if the whitish chalky material is absent in the daytime and diminishes when a Renilla at night is exposed to the electric light. It would seem that Renilla is an excellent form to give answer to the questions proposed above, since the luminous material can be actually seen. Local exposure of non-luminous regions of Renilla to strong illumination would tell us whether the receptors for photogenic nerves are affected by light.

The inhibition of the light of Ctenophores by illumination has been known for a long time. There is no night-day rhythm of luminescence

for Bolina and some other genera, as specimens collected in the daytime will usually luminesce after one-half hour in the dark. The luminous material cannot be easily seen, but the luminous cells lie along the meridians of paddle plates. Bolinas collected in the daytime and stimulated do not luminesce. Neither do they luminesce when thoroughly ground up in a mortar (Harvey 1921 c). This experiment shows that we are not merely dealing with an effect of light on the stimulation mechanism, but that in daylight there is no photogenic material in the luminous cells. Moreover, if a Bolina which has been luminescent on stimulation in the dark is exposed to sunlight for a short time, then brought into a dark room and ground up thoroughly, no luminescence will appear. Hence, illumination causes what photogenic material there is in the cell to disappear.

Moore (1923) has observed that filter paper impregnated in the dark with the luminous tissue of Mnemiopsis loses its power to luminesce by rubbing or treatment with salt solution, after exposure to strong artificial light or sunlight. However, it does not loose its power of luminescence after exposure for thirty minutes to an illumination which will cause an intact Mnemiopsis to lose its power of luminescence in eight minutes The normal animal is therefore much more sensitive to illumination than the luminous secretions. Light not only causes photogenic substance to disappear in cell secretions, but has a marked effect on the luminescence of the entire animal through some nervous mechanism. Illumination of one-half of the animal causes suppression of luminescence in that half alone. Time $\times$ intensity of illumination = a constant, so that a photochemical effect is indicated.

Moore believes the photogenic material is innervated by two kinds of nerves, one from tactile receptors (1) which are distributed only along the paddle plates and quite distinct from the tactile receptors for cessation of paddle plate movement and retraction of oral lobes, which are distributed over the whole of the animals surface; the other nerves from photoreceptors (2) which must have only local connections with photogenic cells since the effect of illumination is only local. The precursor (A) of the luminous material (L) is found in the resting animal in the dark. Stimulation of 1, tactile receptor nerve, converts A to L which then luminesces, while stimulation of 2, photoreceptors, converts A to D, a non-luminescent product. The scheme may be represented as follows:

$$D \underset{\text{dark}}{\overset{\text{illumination}}{\leftrightarrows}} A \underset{\text{rest}}{\overset{\text{excitation}}{\rightleftarrows}} L$$

The observation of Peters (1905) that stimulation hastens the recovery of luminescence in an animal previously illuminated becomes intelligible in the above scheme, since excitation removes A from the system and facilitates the conversion of D into A (recovery in the dark).

There are many luminous forms whose luminescence is not effected by the light. These include the luminous bacteria whose luminescence in continuous and not increased or decreased by ordinary illuminations. Like other bacteria they are injured by strong sunlight or ultra-violet light, although Suchsland (1898) kept luminous bacteria surrounded by water (to protect from heat) for eleven hours in sunlight, without any diminution in intensity of the bacterial luminescence.

Gerretsen (1920) has studied the relation between the bacteria per thousand killed by a quartz 110 volt, 4 to 5 ampere mercury vapor lamp (at 35 cm. distance) and the duration of exposure. The curve is S-shaped with a great increase in the number of bacteria killed between twenty and thirty seconds exposure, and complete destruction in one hundred and twenty seconds exposure. The curve is similar to that obtained for heat in killing bacteria. The ultraviolet light itself must produce the effect directly, since an agar plate exposed to ultraviolet light and then inoculated with the bacteria contains no substances which will injure them or even retard their growth.

Luminescence of the bacteria is not immediately interfered with by exposures to ultraviolet light that kills the bacteria. Bacterial plates exposed to the mercury lamp may glow for six hours before darkening, but the bacteria have lost their power of growth and are eventually killed. This is the opposite of the effect of high temperatures which inhibit the light of bacteria without affecting the growth.

Sometimes a plate partly exposed to ultraviolet was found to glow more strongly over the illuminated area for a short time, later becoming less bright, and finally ceasing to glow. This effect could not always be obtained. It may be due to oxygen or H_2O_2 liberated by the ultraviolet light from the culture medium, as quartz vessels filled with luminous bacteria in absence of oxygen, and consequently non-luminescent, would glow very faintly after exposure to ultraviolet light.

Bacteria exposed to ultraviolet light will respond to temperature changes by a change in intensity of luminescence and will also respond to the addition of glucose by increasing luminescence, although they are destined to die as the result of radiation. Gerretsen takes this fact to mean that luminescence is not a vital process depending on

"living" cells but an enzymic process that continues after the bacteria are killed. He gives the name photogenase to the enzyme forming the photogen[4] and thinks an oxidase (luciferase) oxidizes the photogen. He was able to demonstrate the luciferin-luciferase reaction in Photobacterium javanese but not in Photobacterium phosphorescens.

The question arises whether the long-lasting light after radiation of bacteria with ultraviolet, intense enough to finally kill them, is due to photogen stored up in the bacteria, or whether more photogen is continually being produced. The latter seems to be the case, because on stopping luminescence of bacteria by lack of oxygen and then readmitting oxygen, there is only a slight transient increase in brightness as compared with the original intensity, while the luminescence after exposure to ultraviolet lasts a long time.

LUMINESCENCE AND SYMBIOSIS. For many years Pierantoni (see especially 1923) has held that animal luminescence in general is due to symbiotic bacteria living in the luminous organ and really producing the light. I am convinced that this is not the case in all forms (certainly not in Cypridina) but is the case in some forms (Photoblepharon and Anomalops). I believe the animals in which luminous symbiotic bacteria can be demonstrated are relatively few. Naturally a decision of the question means an intensive study of each luminous form. Generalizations are not possible, although often indulged in.

Zirpolo (1917 to 1922) has studied and grown the bacteria (Micrococcus Pierantoni) isolated from the luminous organ of a Squid (Rondoletia minor), but Montara (1922) was unable to grow bacteria from another Squid (Heteroteuthis dispar) (see Pierantoni, 1922) The great abundance of luminous bacteria in sea water which almost always appear as colonies on dead marine animals makes one cautious in accepting the isolation of symbiotic forms.

It is clamed that the light of the fire-fly and of an ascidian, Pyrosoma, is due to symbiotic luminous bacteria. Vogel (1922) was unable to isolate any bacteria from the fire-fly. A discussion will be found in Büchner's book (1921) and a short review of symbiosis by Nuttall (1923). The controversy is largely morphological and space does not permit a proper presentation here (see Caullery, 1921).

The forms (Photoblepharon and Anomalops) referred to above are fishes, and the complete behavior of the luminous organ is that of

[4] Dubois has called the enzyme co-luciferase, which forms luciferin from a precursor, preluciferine.

an emulsion of luminous bacteria. Besides, the *moving* bacteria can be *seen* in the fresh teased organ, although it was not possible to grow them on artificial culture media (Harvey, 1921 b and 1922 c).

QUALITY OF ANIMAL LIGHT—SPECTRUM. It is well known that the color of animal luminescence is different in different forms and that this represents a real difference in spectral distribution of energy. Bioluminescence spectra are rather broad bands within the visible region showing no indication of line or fluted structure. It is questionable if the limits of these light bands as determined by eye observation have much significance, since with greater intensity, the spectral limits widen. Thus, with Cypridina luminescence, the spectrum, observed with a Zeiss microspectroscope, ordinarily extends from about $\lambda = 0.42\ \mu$ to $\lambda = 61\mu$ while the very brightest light from fragments of luminous gland shows considerable red, extending from $\lambda = 0.650\mu$ to $\lambda = 0.415\mu$. No doubt photographs exposed many hours would show that Cypridina emits wave-lengths over nearly the whole visible region. With increase in temperature the color shifts toward the longer wave-lengths.

The increase in extent of spectra of luminous animals is well seen in some recent photographs I have taken of bacterial luminescence. By eye observation Molisch could only detect wave-lengths between $\lambda = 0.45\mu$ and $\lambda = 0.57\mu$ for various species of bacteria. A 12 hour exposure with a special large aperture spectrograph and one mm. slit-width gave a spectrum extending from $\lambda = 0.587\mu$ to $\lambda = 0.438\mu$ while with an eighty-four hour exposure the limits are $\lambda = 0.667\mu$ to 0.430μ.

These photographs show no trace of ultraviolet radiation, a fact contrary to the finding of Forsythe (Nature, 1910), who obtained, with forty-eight hours exposure, ultraviolet to $\lambda = 0.35\mu$ in bacterial light. No infra-red has been observed in any animal light.

Where two closely allied genera of luminous animals, such as Cypridina and Cypripina, produce light of different colors and their luciferin and luciferase will intermix, the color of luminescence of an intercross is determined by the genus supplying the luciferase.[5]

INTENSITY OF ANIMAL LIGHT. The actual candle power of luminous animals is always small. Various estimations for fire-flies range from $\frac{1}{500}$ to $\frac{1}{4000}$ candle. Pickering (1916), by comparing the flash of the large Jamaican beetle (*Pyrophorus*) at a given distance, with stars of

[5] Recent experiments, not yet published.

various magnitudes arrived at the value 0.004 candle. However, it is not so much the actual candle-power but the intrinsic brilliancy of the light, the candle-power per square centimeter, which is of value in comparing various organisms and other illuminants. Ives (1922) calculated that the Jamaican beetle, whose luminous area is said to be 1.5 cm.^{2}, gave a specific luminous emission of $\frac{0.004 \text{ candle power}}{1.5 \text{ sq. cm.}} \times \pi =$ 0.0084 lumen per cm.^{2}, or a brightness of 0.0084 lamberts.[6] Ives and Jordan (1913) had previously found by direct determination (comparison with a surface whose illumination could be varied) that the glow-worm (larva of Photuris pennsylvanica) gave 0.0144 lumen per cm.^{2} A surface of this brightness 2 meters in diameter in the ceiling of a room 3 meters high would give an illumination on a table 1 meter high of 29 meter-candles, a value satisfactory for general illumination.

Nichols (1922), by means of an optical pyrometer, has determined that the luminescence of moistened fragments of the dried luminous gland of Cypridina show a brightness of 0.0145 to 0.016 lambert. This value compares well with the fluorescence of various substances exposed to the iron spark as an exciting source of fluorescence. Some of Nichols' values for fluorescence are:

	lambert
Rhodamin 6 G, in dilute solution	0.0042 to 0.0120
Fluoresceine, in dilute solution	0.0042 to 0.0052
Potassium uranyl sulphate (solid)	0.0352
Synthetic willemites, solid	0.0125 to 0.0140
Natural willemites, solid	0.0053
Sidot blendes, solid	0.0030 to 0.0109
Calcium sulphide (Balmain), solid	0.0012
Canary glass, solid	0.0073

An incandescent lamp filament has an intrinsic brilliancy of 500 to 630 lamberts and a typical blue sky a brightness of 1 lambert. These values serve to bring out the enormous difference in brilliancy of incandescent lights and luminescences. The luminous paint used in coating watch hands and figures, whose light is a radioluminescence, has a brightness of 10 to 20 microlamberts (10 to 20 $\times$ 10^{-6} lamberts) (Hess, 1922).

Efficiency of Luminescence. By *radiational efficiency* is meant the efficiency by which energy supplied to a light source is transformed into radiant energy (including all wave lengths, whether we can see

[6] One lumen per cm. emission = 1 lambert.

them as light or not); by *radiant luminous efficiency* is meant the ratio of the visible radiation (the radiation which we see as light) × visual sensibility (which takes into account the fact that some colors appear brighter than others, although of equal energy content) to the total radiant energy. The *total efficiency* is the product of the two, radiational efficiency × radiant luminous efficiency.

The radiational efficiency of an incandescent lamp may be high, since 90 per cent of the electrical energy may be transformed to radiant energy. The radiant luminous efficiency is very low, less than 3 per cent of the total radiation being visible radiation even in the best types of incandescent lamp.

In the fire-fly, on the other hand, the radiant luminous efficiency is very high, and this is the efficiency which is usually referred to in speaking of the fire-fly, since we do not know what the radiational efficiency of the fire-fly or any other luminous organism is.[7]

McDermott and Ives (1914) and Ives (1922) have calculated the total efficiency in the fire–fly indirectly. They make the assumption that a man can do muscular work continuously with an output of 1 watt per kilogram, and that the energy output in the glow-worm is, in proportion to weight, the same as in man. Hence, if the glow-worm weighs 0.25 gram and the tissue devoted to light production is $\frac{1}{10}$ of this, 0.025 gram will develop 0.000025 watt. Since the area emitting light is 10 mm.^{2}, the watts per cm.^{2} will be 0.00025. The luminous emission per cm.^{2} they found by direct measurement to be 0.0144 lumen. Hence, $\frac{0.0144}{0.00025} = 57.6$ lumens per watt are produced by the glow-worm. As 640 lumens per watt corresponds to 100 per cent efficiency, $\frac{57.6}{640} = 0.09$, nine per cent total luminous efficiency seems to be attained.

Karrer (1918), from estimates of efficiency of various transformations by living things, comes to the conclusion that the efficiency of light production in the fire-fly is 19.4 per cent.

One cannot help but feel that calculations based upon the efficiency of totally different processes than light production and in totally different animals can give us very little if any information regarding the efficiency of the fire-fly luminescence itself.

[7] In my book, *The Nature of Animal Light*, the divisor in the last column of table 6, p. 60, should be total energy input instead of total radiation. The fire-fly should not be included in the table.

Two calculations of the efficiency of inorganic luminescences are based upon actual measurements. Hess (1922) reports that 0.1 mgm. of radium mixed with 1 gram of ZnS will give a luminous surface of 1.5 sq. inches (9.68 sq. cm.) whose brightness is 10 microlamberts. Ten microlamberts correspond to 10×10^{-3} lumens per centimeter. Over a surface of 9.68 cm. this is 96.9×10^{-3} lumens. Lumens $\div 4\pi$ = mean spherical candle power or approximately 0.8×10^{-5} candles.[8] The luminescence of the ZnS is due to the α particles, whose energy can be derived from the heat-production of radium. The α particles *alone* produce 124 gram-calories per hour. Hence 0.1 mgm. radium gives 0.00000348 calories per second. Multiplied by 4.18[9] this equals 0.0000144 watts and $\frac{0.0000144}{0.00001} = 1.44$ watts per candle. A tungsten lamp consumes 1 watt spherical candle, As $\frac{96.8 \times 10^{-6} \text{ lumens}}{14.4 \times 10^{-6} \text{ watts}} = 6.7$, 6.7 lumens per watt is attained, and as 640 lumens per watt is 100 per cent efficiency, the efficiency of radioluminescence is around 1 per cent.

Adams (1924) has determined the efficiency of the luminescence of phosphorus. When nitrogen is bubbled through a solution of phosphorus in cotton-seed oil the gas becomes saturated with P_4 vapor and luminesces in contact with air. From the rate of flow of gas and the vapor pressure of phosphorus, one can calculate the mols of P_4 per second oxidized. From the heat of combustion of P_4 one obtains the calories per phosphorus oxidized. Multiplied by 4.18[9] we have the watt-second input. The brightness of the greenish phosphorus luminescence by direct measurement proved to be 21.5×10^{-3} lamberts over an area of 0.145 cm. This corresponds to 3.1×10^{-3} lumens. Since light emitted in all directions by the transparent flame will be 4 times that emitted by a disc at right angles to its surface, $3.1 \times 10^{-3} \times 4 = 12.4 \times 10^{-3}$ lumens emitted by the flame. Consequently $\frac{12.4 \times 10^{-5} \text{ lumens}}{11.35 \times 10^{-3} \text{ watts}}$ or about 0.001 lumen per watt or 0.015 per cent efficiency is attained.

If the efficiency of other chemiluminescences proves to be as low as this, the commercial future of chemiluminescence seems doubtful, to say the least. What we really need in the case of luminous animals

[8] Hess gives 1×10^{-4} candles, since 4π is neglected. This reduces his efficiency from 0.144 watt to about 1.44 watt per candle.

[9] One gram-calorie = 4.18 watt-second or 4.18 joules. Joule = 10^7 ergs.

is a determination of the heat of oxidation of luciferin, together with the light produced per calorie, in order that the efficiency can be calculated. Some work now in progress in my laboratory may enable the efficiency for Cypridina to be stated in the near future.

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CONTENTS OF VOLUME XV, No. 6, 1921

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PAGE

LXXXI. Note on the Fermentation of i-Inositol. By J. A. HEWITT and D. B. STEABBEN..... 665
LXXXII. The Metabolism of Carbohydrates. Part II. On the Possible Occurrence of Stereochemical Changes in Equilibrated Solutions of Reducing Sugars Introduced into the Circulation. By J. A. HEWITT and D. H. DE SOUZA..... 667
LXXXIII. A Contribution to the Study of the Interconversion of Carbohydrate and Lactic Acid in Muscle. By D. L. FOSTER and D. M. MOYLE. (With one figure)..... 672
LXXXIV. Blood Enzymes. I. On the Occurrence of Maltase in Mammalian Blood. By A. COMPTON..... 681
LXXXV. Urochrome as a Derivative of Chlorophyll. By H. E. ROAF..... 687
LXXXVI. The Estimation of Carnosine in Muscle Extract. (Preliminary Note.) By G. HUNTER..... 689
LXXXVII. A Study of the Combined Action of Raw Cow's Milk and Orange-juice as Antiscorbutic Substances. By S. WRIGHT. (With two figures)..... 695
LXXXVIII. The Origin of Milk Fat, and its Relation to the Metabolism of Phosphorus. By E. J. SHEEHY (With one figure)..... 703
LXXXIX. Studies of the Coagulation of the Blood. Part I. Some Physico-Chemical Aspects of Coagulation. By J. W. PICKERING and J. A. HEWITT..... 710
XC. The Distribution of Carnosine in the Animal Kingdom. By W. M. CLIFFORD..... 725
XCI. A Note on the Physiology of the Ship-worm (*Teredo norvegica*). By C. R. HARINGTON. (With one figure)..... 736

INDEX..... 742

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Contents of Volume V, 1925

J. J. ABEL: The Pituitary
A. S. LOEVENHART: The Relation of Physiological Oxidations to Functional Activity
CARL VOEGTLIN: Pharmacology of Arsphenamine and Related Compounds
STANLEY COBB: Muscle Tonus
C. G. BULL: Bacteriophages
V. J. HARDING: Metabolism in Pregnancy
K. K. KOESSLER: Putrefactive Amines
E. P. CATHCART: Influence of Muscle Activity on Protein Metabolism
A. R. RICH: Formation of the Bile-Pigments
J. A. DETLEFSEN: Inheritance of Acquired Characteristics
E. J. COHN: Physical Chemistry of the Proteins
R. GESELL: The Regulation of Respiration
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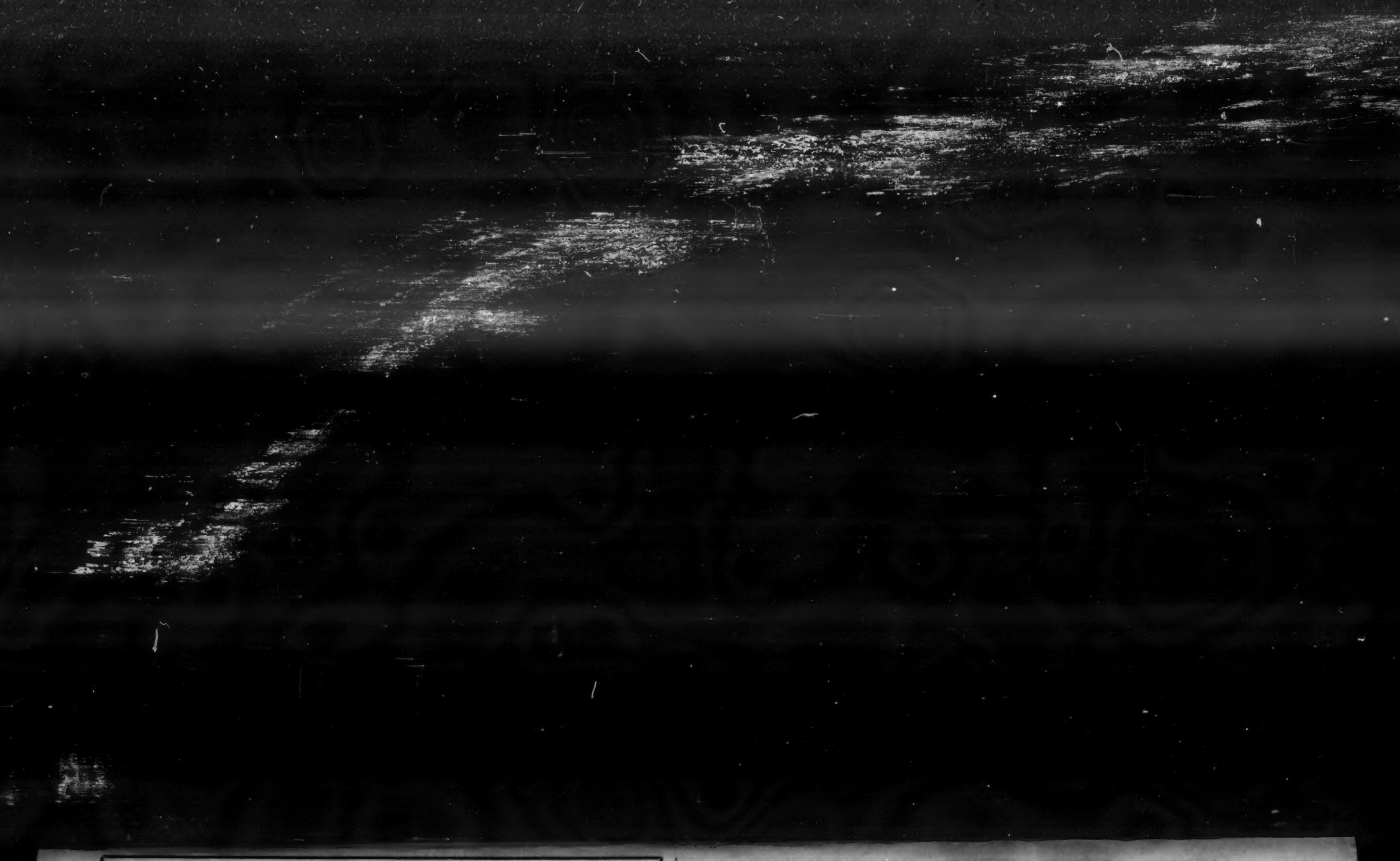
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